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# PROCEEDINGS of the 35th Southern Pasture and Forage Crop Improvement Conference

June 13-14, 1978 Sarasota, Florida





#### **PROCEEDINGS**

OF THE

#### 35TH SOUTHERN PASTURE AND FORAGE CROP

IMPROVEMENT CONFERENCE

June 13-14, 1978 Sarasota, Florida

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#### PLANT COMMUNITIES OF PENINSULAR FLORIDA

#### By James A. Wolfe

In spite of its relatively youthful geologic age, peninsular Florida has a diverse and distinctive flora. A subtropical climate is favorable for the survival of many kinds of native and exotic plants. Subtropical or peninsular Florida is the part of the state from the Gainesville vicinity southward. It roughly corresponds to the hyperthermic region as used in soil taxonomy (1,3).

While climate has been a dominant factor in determining the rich flora of Florida, the influence of soils has also been great. With few exceptions, the parent material for soils of peninsular Florida is marine deposits of Pleistocene age. The landscape is characterized by a series of former shoreline ridges and marine terraces that were formed during interglacial periods when the sea level was higher than at present. The highest areas, being the first to emerge from the sea, have undergone more alteration than the lower, nearly level terraces or flatwoods. The older Central Highlands, no longer resembling a terrace, have karst topography characterized by numerous lakes and depressions resulting from the collapse of solution caverns in the underlying limestone. Recent or Holocene surfaces are conspicuous along the seashore and major streams and in marshes and swamps where organic matter has accumulated. Recent geologic deposits are most apparent in coastal areas where changes occur to varying degrees with each wave, tide, or tropical storm. Under the stabilizing influence of plants adapted to these areas, marine sediments accumulate and land is formed. The nature of the resulting soils is closely related to the kinds of deposits. Sands and broken shells accumulate in beaches and adjacent barrier dunes producing sandy soils. In the shelter of lagoons and tidal inlets, finer sediments accumulate.

Vegetation in coastal areas shows zonation with different stages of primary succession related to progressively older deposits. On barrier dunes, conspicuous plants are sea-oats (Uniola paniculata), beach morning-glory (Ipomoea pes-caprae), and bitter panicum (Panicum amarum). Saw palmettos (Serenoa repens) become established shortly after the rhizomatous pioneer plants stabilize the shifting sands. Live oaks (Quercus virginiana) and other species of trees and shrubs eventually become established and create dense thickets. The climax plant community is a hardwood hammock with many epiphytes attached to the branches of spreading live oaks.

Mangrove swamps are very important as spawning and feeding grounds for fish and shellfish, but they also play an important role in land formation. Red mangroves (Rhizophora mangle), which have prop roots extending out into the water, are the pioneer plants. As sediments accumulate and land builds up, succession proceeds through stages of black mangroves (Avicennia nitida), white mangroves (Laguncularia racemosa), and eventually other plants. The southern tip of the peninsula has extensive mangrove swamps. Toward the north they are eliminated by freezing and are replaced by tidal marshes where the vegetation is mainly smooth cordgrass (Spartina alterniflora), marshhay cord-

grass ( $\underline{S}$ . patens), black needlerush ( $\underline{Juncus}$  roemerianus), and seashore salt-grass ( $\underline{D}$ istichlis spicata).

In the interior of the peninsula, the wetlands, flatwoods, and sand ridges have distinctive types of vegetation. The wetlands have very poorly drained soils that are commonly organic (muck) or mineral soils with a high organic matter content. The flatwoods have poorly drained, sandy soils. The water table is near the surface during the summer rainy season, but during prolonged periods of low rainfall these soils become extremely dry. Soils of the flatwoods are characteristically Spodosols—formerly called ground—water Podzols—except in areas with shallow, porous limestones in the extreme southern tip of the peninsula. The sand ridges have freely drained soils and are of two types. Karst ridges are on the Central Highlands, and former shoreline ridges run almost parallel to the ocean along the lower marine terraces.

The wetlands are swamps dominated by woody plants and marshes dominated by grasses and grasslike plants. Cypress swamps, deciduous hardwood swamps, and evergreen hardwood swamps (bay swamps) are widely distributed along streams and in depressions. The most extensive marshes are in the Everglades region, but they are common along streams and in ponds in other parts of the state. Many of the marshes are dominated by sawgrass (Cladium jamaicense), pickerelweed (Pontederia cordata), or other herbaceous hydrophytes. Some marshes are dominated by maidencane (Panicum hemitomon), an excellent forage species.

Other grasslands with poorly drained soils occur in areas that are covered by water for a shorter time than the very poorly drained marshes. This type of wet grassland sometimes surrounds marshes but is more common along major drainage systems and in sloughs in flatwoods areas. Wet grasslands are more extensive toward the tip of the peninsula. Broad areas of nearly treeless wet prairie are in the vicinity of Lake Okeechobee and the Everglades, and smaller areas are along the St. Johns River. Many of these areas are artificially drained, but before they were drained a sheet of water covered the ground for 2 to 7 months after the summer rainy season began. These grasslands are naturally adapted to grazing, and in many areas they have been converted to improved pasture. Blue maidencane (Amphicarpum muhlenbergianum) and chalky bluestem (Andropogon capillipes) are important native forage grasses. With prolonged heavy grazing, however, sand cordgrass (Spartina bakeri), wiregrass (Aristida stricta), and broomsedge bluestem (Andropogon virginicus) often increase and become dominant.

The flatwoods are broad, nearly level marine terraces. These pine-palmetto communities are the most extensive of the natural communities of central and southern Florida. The landscape is an open forest of slash pine (Pinus elliottii) with longleaf pine (P. palustris) and pond pine (P. serotina) being numerous in some localities. The understory is commonly a dense growth of saw palmettos. Many flatwoods areas are used as native range. Wiregrass commonly is the most abundant grass; however, in areas that are not overgrazed, more palatable grasses such as chalky bluestem, creeping bluestem (A. stolonifer), and lopsided indiangrass (Sorghastrum secundum) are important forage species. These areas are frequently chopped or burned to control saw palmettos and other undesirable plants that compete with the more desirable grasses. This community has a long history of natural and man made fires. It is considered to be a fire subclimax community. If fire or other disturbances were eliminated for a very long time, these areas would eventually develop other types of communities.

The sand ridges have freely drained soils. The water table is ordinarily below a depth of 20 inches and in most areas it is much deeper. The two most widespread types of vegetation are the sand pine scrub (sand pine - scrub oaks) and the longleaf pine - turkey oak communities. Both communities are fire subclimaxes. Without periodic burning, they would develop into some type of hammock. The sand pine scrub type occupies the most infertile areas of the sand ridges. In addition to sand pine (Pinus clausa), this community is characterized by scrub oaks: myrtle oak (Quercus myrtifolia), sand live oak (Q. virginiana var. geminata), and Chapman oak (Q. chapmanii). The soils are highly leached and very droughty, and white sand shows through the sparse groundcover in many areas. Sand pine scrub communities are common on relict dunes of former shoreline ridges and in the driest areas of the Central Highlands, especially in the Ocala National Forest. Longleaf pine - turkey oak communities are widely distributed in the Central Highlands. Agriculturally developed areas are in improved pasture and citrus. Where natural vegetation remains, the landscape is an open forest characterized by longleaf pine, turkey oak (Q. laevis), and some blue jack oak (Q. incana). Saw palmettos are scattered and the ground cover is commonly wiregrass. While soils are not so highly leached as those of the sand pine scrub, they are naturally infertile and droughty.

Several types of hammock are in Florida. A hammock is a type of plant community dominated by broadleaf evergreens, for example, large, spreading live oaks. Some hammocks are climax communities and some are preclimaxes. The total acreage for hammock communities is small due to fire or other disturbances. Without disturbance, most areas, except those with very poorly drained soils, would apparently develop some type of hammock. Tropical hammocks characterized by gumbo-limbo (Bursera simarouba), strangler fig (Ficus aurea), poisontree (Metopium toxiferum), Jamaica dogwood (Piscidia communis), marlberry (Ardisia escallonioides), and other tropical species are in the extreme southern part of the state. Other hammocks occupy hydric, xeric, or mesic sites throughout the rest of the peninsula. The hydric or wet hammocks are common along streams and sloughs. They have an abundance of cabbage palm (Sabal palmetto) as well as live oak and laurel oak (Q. laurifolia). Xeric or dry hammocks are on the sand ridges and are the result of exclusion of fire from longleaf pine - turkey oak and sand pine scrub communities. Two indicator species of the mesic hammock are southern magnolia (Magnolia grandiflora) and American holly (Ilex opaca), and the mesic hammock is sometimes called the Magnolia-Ilex climax (2). Large spreading live oaks and other large trees are also present. The mesic hammock is considered to be the climatic climax community for central Florida. It is the final stage in succession and its acreage is comparatively small because of many past disturbances. Mesic hammocks occur on many freely drained soils, but succession is more rapid in soils with favorable moisture conditions. Hammocks have little value for forage but can provide shelter for livestock and wildlife. Because of their attractive setting, they are desired for community development.

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#### IMPROVED FORAGES

# By O. Charles Ruelke

Improved forages have been a key factor in the initial survival and later development of improved livestock in Florida. Without improved forage species and forage management systems it would be impossible to support improved breeds of livestock, especially high producing dairy cows, unless a major proportion of the feed requirement is shipped in to Florida. In some countries, like the British Isles and New Zealand, most if not all of the feed requirements come from improved forages. Florida has favorable climate, available land and many improved forage species to choose from to meet the animal feed requirements. Our task as research workers is to find the best species adapted to particular sites, and manage them in such a way as to provide the feed requirements at a minimum cost.

The environment of Florida is extremely variable with a temperate climate in northwest Florida to a sub-tropical climate in south Florida, and tropical storms to extreme dry conditions. Some of the most fertile organic muck soils are found in Florida, as well as some of the most sterile sand dunes which occur along Florida's coasts. Florida is a world source of phosphate fertilizer deposits as well as completely sterile sand that can be used directly from the field to do nutrient deficiency research.

Likewise, Florida's forage species range from the poorest, unpalatable, indigestible native species like sedges and rushes to the most productive, palatable and digestible species known, like white clover and ryegrass. Acreages of improved forages are shown in Table 1. The seasonal distribution of forage species throughout north, central and south Florida is shown in the grazing calendar, Figure 1. This grazing calendar includes only a few of the many possible forage species which can be grown in Florida. Herein is one of the problems, or opportunities, depending upon how you look at it. With so many differen species to choose from, it is difficult to develop breeding and management research on all of the species which have potential for forage. Likewise, with so many different species to choose from it is possible to select a cultivar for a particular ecological niche.

In Florida, several different procedures for obtaining improved forages have been very successful. 'Pensacola' bahiagrass, an improved bahiagrass cultivar, was selected from bahiagrass plants which were found along the docks near Pensacola, Florida, where bananas, which were packed in hay, were unloaded. Today, bahiagrass is the most extensively grown improved grass in Florida.

'Pangola' digitgrass, which was introduced from the region of the Pongola river in South Africa, is now one of the most extensively grown grasses in central and south Florida. This important improved grass is believed to have originated as a natural hybrid in Africa. Its forage potential was not realized there, but thanks to the keen eye and imagination of several Florida scientists, it has been vegetatively propagated and grown in Florida and distributed throughout sub-tropical and tropical regions all over the world. It is interesting to note that all of the 'Pangola' cultivar of digitgrass that exists in

Table 1.--Acreages of grasslands in Florida\*

ltems	1974 acres	Projected 1980 acres	Acreages 1985 acres
Total Grassland	12,167,000	11,967,000	11,767,000
Range pastures Woodland pastures Improved permanent grass pastures Digitgrasses Bahiagrasses Bermudagrasses Miscellaneous Grass-legume	3,969,000 4,698,000 3,125,000 628,000 2,245,000 186,000 66,000 (447,000)	3,738,000 4,600,000 3,220,000 648,000 2,312,000 192,000 68,000 (500,000)	3,383,000 3,500,000 3,437,000 691,000 2,469,000 204,000 73,000 (600,000)
Temporary <sup>2</sup> Summer annuals Millet Sorghum X sudan Alyce Clover Indigo Miscellaneous	688,000 221,000 65,000 36,000 50,000 50,000	801,000 245,000 75,000 45,000 60,000 50,000 25,000	955,000 280,000 90,000 60,000 70,000 50,000 30,000
Winter Annuals Rye Wheat Oats Ryegrass	467,000 101,000 12,000 28,000 326,000	546,000 104,000 13,000 29,000 400,000	655,000 111,000 14,000 30,000 500,000
Silage Corn Sorghum	23,000 16,000 7,000	29,000 20,000 9,000	42,000 30,000 12,000
Hay	(188,000)	(207,000)	(226,000)

Most of these acreages included in improved permanent grass pastures; overseeded with winter growing legumes or harvested for hay.

From: Ruelke, O. C. and G. B. Killinger, 1977. Chapter II. Forage and Pastures from Beef Cattle in Florida Bull 28: Fla. Dept. of Agric. and Consumer Service and Inst. of Food & Agric. Sci. pp 143-164.

 $<sup>^{2}</sup>$ About 50 percent of temporary pasture acreage is double cropped.

<sup>\*</sup>Data prepared for commodity report of the Forage, Range and Pasture Committee presented to Agricultural Growth in an Urban Age Conference, Feb. 11, 1975.

Figure 1.--Grazing Calendar for Florida

GRAZI	NG	CAL	FND	٨R
GINALI	110	CAL		AIL
	WINTER	CDDING	CHAMED	FALL
			SUMMER	
	JAN-MAR	APR-JUN	JUL-SEPT	OCT-DEC
N	ORTH	FLORID	A	
BAHIAGRASS		1		
BERMUDAGRASS		S. P. S. S.		
DALLISGRASS		100		
FESCUE	2	对效等。		
WHITE CLOVERS		realiza es		
OTHER CLOVERS				
SMALL GRAINS	a Fillyaria			
RYEGRASS	. F K per			
MILLET & SORGHUM				
CE	NTRAL	FLORID	A	
BAHIAGRASS		a constant		
PANGOLAGRASS		The state of		
BERMUDAGRASS		Sall alexander		
WHITE CLOVER		A STATE OF THE STA		
GRASS CLOVER				
ALYCECLOVER				
SMALL GRAINS	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			
RYEGRASS	1.24 4 184 6			
MILLET & SORGHUM				t\
	HTUOS	FLORI	DA	
PANGOLAGRASS		The second second	Bath The Land	
BAHIAGRASS	1,50		Page 2 September 1	the state
BERMUDAGRASS				
ST AUGUSTINEGRASS	12	-	THE STATE OF THE	-
PARA-CARIBGRASS			a paredi i -	
WHITE CLOVER	THE WALL			
MISC. LEGUMES				

From: Ruelke, O. C. and G. B. Killinger 1977. Beef Cattle in Florida. Bul. 28. Fla. Dept. of Agriculture and Consumer Services and Institute of Food and Agricultural Sciences. Ch. 11. pp 143-164.

pastures throughout the world originated from a clone evaluated in the plant introduction nursery of the Florida Experimental Station at Gainesville, Florida, and recent plant explorations into Africa have not been able to find the genetically identical plant in existence in the region where it was originally found.

Bermudagrasses are also extremely important as improved forages for Florida and all of us here are familiar with the outstanding work of Dr. Glen Burton and the breeding of 'Coastal', 'Coastcross l', and more recently 'Tifton 44' bermudagrass. More recent introductions from Africa and Europe, and incorporation of these in breeding for higher production, higher digestibility, and better adaptation to eliminate stress of cold and drought, have resulted in far superior cultivars of bermudagrass for Florida and all of the world.

Many other introduced and improved genera of grasses have contributed to the improved forage supply of specific areas of Florida. These include grasses of the genera Axonopus, Brachiaria, Cenchrus, Chloris, Echinecoloa, Hemarthria, Lolium, Panicum, Paspalum, Pennisetum, Secale and Stenotaphrum.

Legumes have also played a very important role in the forage program in Florida. It has been demonstrated that you cannot grow white clover successfully in Florida, and also that you can grow white clover successfully in Florida. Choice of site and management research brought out the keys to success. In early work on sites favorable for grazing white clover, with proper management, average weaning percentages increased from 63%, on fertilized grass pastures, to 81% on grass clover pasture with no nitrogen applied. In central Florida, early work, Table 2, has shown higher calf weaning percentage, calf weaning weights, slaughter grades and calf production per cow and per acre from improved grass clover pastures than from native or native and improved grass pasture.

In south Florida, because of its subtropical wet climate, there has been an excellent opportunity to evaluate many of the tropical legumes, as well as to breed improved cultivars. This work has led to the use of many new genera of legumes including Aeschynomene, Alysicarpus, Arachis, Cajanus, Centrosema, Clitoria, Desmodium, Glycine, Indigofera, Leucaena, Lablab, Macroptiluim, Pueraria, Vigna and Zornia.

Finally, no improved forage program would be complete without research on preservation of forage as hay, haylage, dehydrated forage, pellets and silage. Cooperative research between agronomists, animal scientists, dairy scientists, and animal nutritionists have made it possible to devise systems for handling and evaluating improved forages and determining the economic returns.

In closing may I take this opportunity to welcome you to our state. We hope your visit here will stimulate and exchange new ideas regarding the use of improved forages.

Table 2.--Average production, supplemental feed, pasture treatement per cow season over a five-year period grazing native, native plus improved and all improved grasses and clover pastures \*

Pasture System	Native	Combination Native & Improved	All Improved with Irrigated Clover
Number of acres	772	388	107
Production Data No. cow seasons Av. wt. cows (lb.) Calf weaning % Calf weaning wt, (lb.) Slaughter grade Calf production/cow (lb.) Calf production/acre (lb.)	303 889 63 380 9 241	(73 Improved) 295 942 75 457 10 340 52	(27 Irrigated) 300 1017 81 504 11 406 228
Supplemental feed cow (1b)			
Hay Cottonseed meal Citrus meal Common salt Mineral	555 52 52 35 39	43 65 31 34 37	280 - - - 17
Pasture Treatment			
Fertilizer/cow (lb.) Complete fertilizer Ammonium nitrate 0-8-24 Muriate Lime	- - - -	237 102 169 - 339	500 133 133 25 500
Renovation (acres) Hubam seed (lb.) Electricity KWH	- - -	0.34 5 -	0.32 - 217

<sup>1</sup> Grades: 9 - Low Good; 10 - Good; 11 - High Good

<sup>\*</sup>Peacock, F. M., E. M. Hodges, W. G. Kirk, and M. Koger. 1967. <u>Cow-Calf Program on Native, Improved and a Combination of Native and Improved Pastures.</u>
Beef Cattle Short Course, University of Florida, Gainesville, Florida.

#### FLORIDA'S RANGE RESOURCE: A PRIMARY SOURCE OF FORAGE

#### By R. S. Kalmbacher

Florida's native-forage pastures cover 3.85 million hectares  $(\underline{1})$  (including grazable woodland) which is seven times greater than the land area of the state of Delaware. In terms of value for wildlife habitat, water recharge and esthetics this represents an important resource, but consideration to the production of indigenous forages which historically has been supporting cattle for more than 400 years makes native range an integral part of Florida's livestock industry.

When people think of rangeland, they most often relate to the western areas, unaware of Florida's resource. Unlike the rangelands west of the 100th meridian, Florida has abundant rainfall which permits greater annual forage production. Short and tall grass prairie rangelands of the western U.S. receive annual rainfall of 25 to 100 cm but in Florida rainfall frequently exceeds 140 cm annually.

Much of Florida's productive rangeland is on the central and southern peninsula. The soils, with the exception of peats and mucks, are sandy and low in fertility. Even though they respond to fertilization  $(\underline{16})$ , rangelands do not receive lime or fertilizer.

Approximately 332 native grasses occur in Florida  $(\underline{23})$ , but only 10 to 15 produce most of the forage consumed by cattle. These grasses of economic importance are characteristic of the site where they grow. A "site" is a natural plant community adapted to rather broad - but distinctive - environmental conditions. Some of the major sites and their important grass species are listed in table 1. Because the flatwood site is the largest type, it has received the greatest amount of research attention in the past 20 years. The discussion of Florida's range management that follows will deal almost exclusively with flatwoods grass species. The purpose of this paper is to familiarize you with Florida range through a review of the literature since the 1940's.

A major tool used in range management has been fire or prescribed burning. Flatwoods are generally burned every two or three years to remove that hazard of accumulated fuel and to improve forage quality. After a burn pineland threeawn (Aristida stricta), also referred to as "wiregrass", regenerates rapidly. In a south Florida study by Hilmon and Lewis (4) this grass comprised 95% of the total herbage three weeks after a February burn. Pineland threeawn yields at three weeks after burning equaled 74 kg of dry matter/ha but increased to 3360 kg/ha after one and one-half years. Research at the Range Cattle Station at Ona, Florida (2) indicated that crude protein in pineland threeawn varied from 10% shortly after burning to  $1\frac{1}{2}$  to  $2\frac{1}{2}$  percent in mature forage. Other researchers have observed a similar drop in forage quality (3, 5, 6, 7, 9, 10, 11, 12, 14, 15) which is associated with a decline in this species' consumption by cattle four to six months after burning. After pineland threeawn maturity, cattle prefer the more palatable tall grasses viz. bluestems, indiangrass and Paspalums (6). Generations of Florida cattlemen

TABLE 1.--Florida's major rangeland sites with their edaphic and vegetative characteristics $^{-1}/$ 

Flatwoods nearly level, deep-sandy creeping bluestem (Schizachyrium stolonifer) somewhat poorly drained soil due to pan at 60 cm.  Slough level, soil poorly drained and covered with shallow water during summer during summer  Sandhill nearly level to strongly soils sea level, generally near sea level, generally near sea level, generally near sea level, generally near sandy-clay loans.  Flatwoods  Creeping bluestem (Schizachyrium stolonifer)  Plue maidencame (Amphicarpum muhlenbergianum) chalky bluestem (Andropogon capillipses)  Pineland threeawn (Aristida stricta)  Pine maidencame (Andropogon capillipses)  Pineland threeawn (Aristida stricta)  Pine maidencame (Andropogon Longiberbis)  Loothachegrass (Ctenium aromaticum)  Creeping bluestem aromaticum)  Loothachegrass (Ctenium aromaticum)  Loothachegrass (Stipa avenacea)  Loothachegrass (Stipa avenacea)  Loothachegrass (Sipatina patens)  marshhay cordgrass (Spattina patens)  marshhay cordgrass (Spattina patens)  sandy-clay loans.	Type	Site Characteristics	Important grass species
level, soil poorly drained and covered with shallow water during summer nearly level to strongly sloping well to excessively drained, deep-sandy soils sea level, generally near estuaries and satuated by tidal inundation. Soils sandy-clay loans.	Flatwoods	nearly level, deep-sandy somewhat poorly drained soil due to pan at 60 cm.	creeping bluestem (Schizachyrium stolonifer) lopsided indiangrass (Sorghastrum secundum) blue maidencane (Amphicarpum muhlenbergianum) chalky bluestem (Andropogon capillipes) Pineland threeawn (Aristida stricta)
nearly level to strongly sloping well to excessively drained, deep-sandy soils sh sea level, generally near estuaries and satuated by tidal inundation. Soils sandy-clay loans.	Slough	level, soil poorly drained and covered with shallow water during summer	blue-joint panicum (Panicum tenerum) blue maidencane hairy bluestem (Andropogon longiberbis) toothachegrass (Ctenium aromaticum)
sea level, generally near estuaries and satuated by tidal inundation. Soils sandy-clay loans.	Sandhill	e d	creeping bluestem lopsided indiangrass needlegrass ( <u>Stipa avenacea</u> ) lovegrass ( <u>Eragrostis spp.</u> )
	Salt marsh	a s	saltgrass (Distichlis spicata) marshhay cordgrass (Spartina patens) smooth cordgrass (S. alterniflora)

 $<sup>\</sup>frac{1}{2}$  Adapted from Important Native Grasses for Range Conservation in Florida. USDA. - S.C.S., Gainesville, Florida.

have managed rangeland by the philosophy of burn and graze. This led Yarlett  $(\underline{22})$  to conclude that repeated burning and uncontrolled grazing led to a decrease in the tall grasses.

Prior to 1960 the research effort had been devoted to native range as a producer of pineland threeawn. There was nothing in the literature about Florida's native tall grasses. Certainly, ranchers were aware of their existence but not their importance. This recognition came about through a few key people, primarily within the USDA - Soil Conservation Service and Forest Service. Yarlett (23) described some important flatwoods grasses: creeping bluestem (Schizachyrium stolonifer), chalky bluestem (Andropogon capillipes), toothachegrass (Ctenium aromaticum and C. floridanum), lopsided indiangrass (Sorghastrum secundum) and blue maidencane (Amphicarpum muhlenbergianum). Later Yarlett (23) compiled data from field observations on many of the important range grasses and described the distribution, site adaption and superficial habits of growth, development, and reactions to grazing. more detailed work conducted at the SCS plant materials center in south Florida Yarlett and Roush (25) described the above characteristics of creeping bluestem emphasizing its potential. Roush and Yarlett (20) were less descriptive and leaned more toward management when they compared creeping bluestem with chalky bluestem, south Florida bluestem (Andropogon rhizomatus) broomsedge (A. virginicus), and Florida threeawn (Aristida rhizomorophora) and found that creeping bluestem out-yielded the other four grasses. The yields of three of the more desirable range grasses are compared with pineland threeawn in table 2. Unfortunately there has been little consistency or qualification in expressing the yields of the native grasses. Many of the yields reported do not accurately reflect the herbage of value to livestock. example is that yields often represent forage accumulated after several years growth.

Higher yields resulting from a change in the botanical composition due to chopping with tendem-drum type choppers and resting flatwoods range have been reported (16, 18, 24). It seems that overgrazing flatwoods range had eliminated the more desirable tall grasses except under the saw palmettos (Serenoa repens) where cattle could not graze them. Chopping and resting for one to two years allows the more desirable grasses to increase. Lewis (16) noted that dry matter yield on chopped, unfertilized range increased from 900 kg/ha to 2420 kg/ha two to five years following treatment. Moore (18) noted a desirable increase in Andropogons, Panicums and Paspalums and a marked decrease in Aristida species. Two years after chopping, dry matter production was 6050 kg/ha. Yarlett (24) indicated that green weight yields of creeping bluestem increased from 1790 to 6730 kg/ha at three and eleven months after chopping, respectively. Yarlett and Roush (25) stated that creeping bluestem increased from about 200 kg/ha to 4260 kg/ha of air-dried material per hectare 1 year after chopping. In grazeable woodlands these yields increased  $170\,$  to 2240 kg/ha.

Forage quality from Florida's native range generally reflects the low fertility of the native soil. Table 3 contains a summary of some quality parameters reported for various grasses. Most of the work has been done on pineland threeawn. Some data are available from fairly recent quality estimates such as  $\underline{\text{in } \text{vitro}}$  or Van Soest analyses.

TABLE 2.--Yield of four Florida native range grass species

Species	Yield	Qualification	Reference
Pineland three-awn (Aristida stricta)	70 kg/ha (DM) 3360 kg/ha (DM) 1350 kg/ha (fresh wt) 450 kg/ha (DM) 560 kg/ha (DM) 1600 kg/ha (DM)	3 wks after burn 20 mos. after burn annual production 4 mos. after burn 7 mos. after burn 19 mos. after burn	Hilmon & Lewis (4) Hilmon & Lewis (4) Roush & Yarlett (20) Hughes (7) Hughes (7)
Creeping bluestem (Schizachyrium stolonifer)	5000 kg/ha (air dry) 4060 kg/ha (air dry) 1790 kg (fresh wt.) 6730 kg (fresh wt.) 1040 kg/ha (DM) 330 kg/ha (DM) 620 kg/ha (DM)	not stated 1 yr. after chopping 3 mos. after chopping 11 mos. after chopping June to Sept. 0ct. to Jan. Feb. to May	Yarlett (22) Yarlett (25) Yarlett (24) Yarlett (24) Kalmbacher (unpublished Kalmbacher (unpublished
Chalky bluestem (Andropogon capillipes)	490 kg/ha (fresh wt.)	annual production	Roush & Yarlett (20)
Maidencane (Panicum hemitomon)	8970 kg/ha (fresh wt.) cut in Sept.	cut in Sept.	Yarlett (23)

TABLE 3.--Quality and chemical composition associated with five Florida native-rangegrass species

		Crude							
Species	In vitro	$\Box$	MQ %	Cellulose Lignin %	Lignin %	Ъ%	Ca %	Qualification	Reference
Pineland threeawn ( <u>Aristida stricta</u> ).		10.0 3.3 2.8 3.7 4.6	15 54 74 36 35	38 8	15 16	0.05 0.03 0.01 0.08 0.09	0.06 0.06 0.43 0.44 0.46	after burn 5 mos. after burn 12 mos. after burn unburned burned annually mowed annually	Davis & Kirk (2) Hilmon & Lewis (4) Hilmon & Lewis (4) Kirk et al. (14) " " " Lewis (16)
	43+	3.2	37			•	•	April October	
Creeping bluestem (Schizachýrium stolonifer)	54+ 40+ 36+ 33+	10.2 3.2 5.1 4.7	31					April <sup>8</sup> October June August October	Lewis "  Kalmbacher (unpub.)  "
Maidencane ( <u>Panicum hemitomon</u> )		20.4				0.25	0.21	May September (native)	Wichman & Fox (21)
Chalky bluestem (Andropogon capillipes) Indiangrass (Sorghastrum secundum)		5.9	38.8	32.1	13.5	3.5 0.06	0.09	3 mos. after burn 2 mos. after burn	Lewis Lewis

<sup>+</sup> IVDMD

<sup>#</sup> IVOMD

<sup>\$</sup> Analysis includes other bluestem grasses.

### Grazing Management and Animal Response

It has been considered that approximately 6 hectares of native range is necessary to support a single cow weighing 290 to 410 kg. Kirk et al. (13) found that unsupplemented cow/calf herds grazing unburned pineland threeawn range had a 61% calf crop and calf production was 19.8 kg/ha when stocked at one cow per 8.1 ha. When stocked at 1 cow per 6.4 ha on range where one half the experimental area had been burned and cattle received molasses for a 135 day period, calf crops were 72% with calf production at 23.3 kg/ha. The mean 205-day calf weights on these two treatments were 172 and 176 kg, respectively. Hughes (7) reported pineland threeawn utilization at 63%, 52% and 46% with stocking rates of one cow per 6.1 ha, 8.9 ha, and 14.6 ha, respectively, seven months after burning.

The most common and practical method of management is to combine native and improved pastures to provide the best utilization of native forage and maintain the breeding herd in good productive condition. When using the premise of one cow/6 ha, Jones et al. (8) at the Range Cattle Station at Ona replaced 3 to 4 ha of native range with 0.4 ha of improved pasture. Cattle had access to native range and rotationally grazed pastures with no supplemental feed. During this five year study cows of breeding age averaged an 80% calf crop and 193 kg weaning weight.

During a 5-year study at Ona ARC to compare cow-calf production from native, combination of native and improved, and improved pastures Peacock et al  $(\underline{19})$  reported birth and weaning rates on the native pasture were 65% and 63%, respectively, compared to 78% and 75% for the combination and 83% and 81% for the improved system (table 4). Average 205-day weights were 173 kg, 196 kg and 208 kg, respectively with market grade values of 8.7, 9.7 and 10.9 listed in order of increasing intensification. These differences demonstrated the annual distribution of forage quantity and quality.

A system frequently used today which may optimize the annual distribution of forage quality and quantity of native and improved pastures is to place cows on native pastures after weaning in the fall. Calves are born on the range supplemented with mineral and protein, then cows and calves are moved to freshly fertilized, improved pasture for the spring and summer. Such a system is intuitive: there is no research to support it as the most nutritionally or economically expedient in terms of livestock production.

#### Economics

Adequate data on costs and cattle production are not available to make an accurate economic evaluation of native pastures. One point can be stressed in an economic discussion about Florida's native range, and that is, that the resource does have the potential of off-setting some of the cash costs of calf production. Management costs on a per hectare basis are lower than those of improved pastures.

#### Summary

The native range represents a valuable resource for Florida cattlemen. Native pastures prior to 1960 were managed for pineland threeawn, but emphasis has shifted to management for the more palatable, higher producing bluestems, maidencane, indiangrass, etc. Research underway within Florida's Institute of Food and Agricultural Sciences (IFAS) involves measurement of the plant

TABLE 4.--Adjusted means for birth, weaning, weaning weight, 205-day weight, market grade and age at weaning of calves from cows on three south-Florida pasture systems. Ona, Agricultural Research Center. 1962-1966

		Pasture System	
	Native	Native & Improved	Improved
Birth rate %	65	78	83
Wean rate %	63	75	81
Weaning weight kg	173	207	229
205-day weight kg	173	196	208
Market grade†	8.7	9.7	10.9
Wean age (days)	209	220	229

<sup>+</sup> From Peacock et al. (19)

<sup># 8,</sup> high standard; 9, low good; 10, good, 11, high good.

response to grazing, saw palmetto control, effect of fire and grazing on the important tall grasses, range rehabilitation, and chemical composition of the more desirable native grasses.

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#### THE FLORIDA DAIRY INDUSTRY

By Barney Harris, Jr.

The dairy industry in Florida is geared to meet state needs for fluid milk, providing approximately 94% of the fluid milk consumed. Milk production in Florida has been increasing at a steady rate since the end of World War II, with notable gains since 1965. Increased production is the result of a steady increase in dairy cow numbers as well as in milk yield per cow. Since 1965, the number of dairy cows in Florida has increased an average of 2% to 3% per year. while the US dairy cow population has been decreasing.

In 1977, Florida had the largest average dairy herd size in the U.S.A., with 400 dairies having an average of 500 cows. Another distinguising characteristic of Florida dairies is their heavy use of use of commercial feeds: Florida dairymen use a higher proportion of commercial feeds than milk producers in any other state. However, through the use of by-product feedstuffs such as molasses, citrus pulp, and fiber feeds such as cottonseed hulls and sugarcane bagasse pellets, the proportion of concentrates, such as corn and wheat are usually below the average for other dairy states.

In penisular Florida, a large majority of the dairies use complete feeds containing cottonseed hulls or sugarcane bagasse pellets. In the northwest or panhandle area of the state, the feedstuffs includ corn silages, hay crop silage and excellent winter pastures. The variation in feedstuffs used is principally due to her size, land fertility, labor problems and land availaility and costs.

Dairymen have shown a greater interest in growing corn silage in recent months. The increased interest has developed primarily as a result of the large fluctuation in the cost of by-product roughages and the desire to use a good roughage. In many cases, a double and triple cropping system is being used to grow forages.

Also, a few Florida dairymen have extended their silage feeding program from a few months of feeding to year round feeding of silage. Year round feeding of silage has certain advantages in that it provides the dairymen with more ease in maintaining a normal milk fat test, a more consistent feeding program, and a source of roughage during the summer months when roughages are frequently expensive. Because of the environmental conditions in Florida, dairymen find that high producing coes can consume up to about 50 lbs of silage during the cool months and 30 lbs during the summer months. Lower producing cows can consume more silage since less grain is needed to meet their energy requirements.

Storage facilities for bulk feeds are available at most dairies. In general, a number of 25 to 30 ton storage tanks are installed at the larger

dairies to handle either a complete feed or certain ingredients plus a premix. Storage facilities for silage in general include upright silos for smaller dairies and bunker or tranch silos for larger dairies.

The feed handling equipment that appears to be the most popular in new dairies for feeding cows is the mixer wagon with scales and the timing system (similar to the Badger feeder). Both systems will deliver measured amounts of feed to dairy cows so that the manager can keep a running account on the amount of feed being consumed. Each system is common in Florida and can be observed at a number of dairies

The feeding area commonly used in the larger dairies includes a milking palor, a feed barn and a bunk for feeding on the outside. The feed barns are paved and, in most cases, contain individual stanchions. Most new barns contain flush systems. Outside feed bundks are usually portable and are moved as frequently as needed. The newer dry-lot installations have paved outside feeding areas with shade.

Herd size in Florida varies from approximately 100 cows to about 6,000 cows. In general the dairies in South Florida tend to be larger (500-1000 cows) while the dairies in the ramining part of the state are smaller but quite variable in size.

Table 1 shows the counties where the largest number of dairies are located. As you may note, primary dairy areas appear to be in the Jacksonville area (Duval, Nassau and Clay counties), the Tampa Bay area (Hillsborough, Pasco, Mantee and Polk counties), the Okeechobee area (Okeechobee, Highlands and Palm Beach counties) and a number of dairies near Mayo (Lafayette county), Marianna (Jackson, Holmes and Washington counties) and Pensacola (Escambia county).

TABLE 1.--Florida counties with ten or more dairies

County	Number of Dairies	County	Number of Dairies
Hillsborough	62	Po1k	14
Duval	27	Jackson	14
Okeechobee	29	Palm Beach	12
Pensacola area	24	Holmes	14
Lafayette	21	Highlands	13
Pasco	15	Marion	12
Manatee	19	Nassau	11

Permanent pastures containing Bermuda, pangola, bahia and native grasses are used quite extensively. This is especially true in many of the herds where adquate pasture land is available and semi-complete feeds are used with a minimum amount of hay. The importance that dairymen place on the permanent pasture as a part of the total feed varies considerably from almost nothing to a maximum of 60-70 pounds of pasture forage per cow per day.

# FORAGE GRASS BREEDING AT THE UNIVERSITY OF FLORIDA

### By K. H. Quesenberry

Historically, the forage grass breeding program at Florida has been concentrated on genetic improvement of perennial tropical grasses. Pangola digitgrass, <u>Digitaria decumbens</u> Stent., an increase from a South African plant introduction, proved to be a top forage producer on sandy flatwood soils of central and south Florida. Utilization of Pangola is limited somewhat by lack of winter-hardiness.

In the early 1960's, a program for genetic improvement of digitgrass was initiated under the leadership of Dr. S. C. Schank. The objectives of this program were improved winter-hardiness, higher digestibility, and possible seed propagation. Dr. Schank has assembled an array of <u>Digitaria</u> germplasm and has made numerous interspecific hybrids in an effort to accomplish these goals. Two cultivars, 'Slenderstem' and 'Transvala' have been released from the Florida <u>Digitaria</u> program. One of Dr. Schank's promising new hybrids (46-2) is significantly higher in digestibility than all other released digitgrass cultivars.

An integral part of the forage improvement program at Florida has been cooperative international evaluation of Florida developed germplasm. This international testing of <u>Digitaria</u> germplasm led to the identification of Pangola Stunt Virus (PSV), a serious disease in several Central and South American countries. Although this virus has not been identified in Florida pastures, it has devastated Pangola pastures in Guyana. The cultivar Transvala is resistant to PSV and was released for utilization both in Florida and internationally.

Dr. A. E. Kretschmer at the Agricultural Research Center, Ft. Pierce, has tested several <u>Digitaria</u> introductions at this more southern location. A new cultivar, 'Tiawain' digitgrass (<u>D. pentenzii</u>) will be released in 1978. This cultivar produces somewhat more dry matter in the cool season and is perhaps better adapted for hay production than Transvala.

A program for the improvement of guineagrass, Panicum maximum Jacq. was initiated by Dr. R. L. Smith in 1970. Guineagrass is an important forage in many areas of the humid tropics; however, most "land" varieties are apomictic. Dr. Smith has identified a source of sexual germplasm and has produced several hybrids using apomictic males and sexual females. Some of these hybrids can be stabilized as apomictic lines and several have been tested for forage potential. Guineagrass usually will not perenniate in north-central Florida and its use will likely be limited to south Florida and the tropics.

Dr. Smith has devoted much of his research in the past three years to studying the potential of associative N<sub>2</sub>-fixation by grass-bacteria systems. One breeder line of guineagrass has shown a yield response to inoculation with the bacteria Azospirillum brasilense. As a part of this research, Dr. Smith has begun a study of the forage potential and nitrogen efficiency of switchgrass, Panicum virgatum.

Limpograss, Hemarthria altissima (Poir) Staph and Hubb, was first intro-

duced into Florida in 1964. Three introductions, P.I. 299993, 299994, and 299995 were increased and tested over a ten year period by various forage workers throughout the state. Limpograss is best adapted to the wet flatwood soils of central and south Florida, although it has been grown successfully at Jay in west Florida. It has superior winter-hardiness to most <u>Digitaria</u> introductions. An estimated 6000 to 8000 ha are currently planted to limpograss. In 1978, three cultivars ('Redalta', 'Greenalta', and'Bigalta') were officially released as direct vegetative increases, respectively, of the above three introductions.

A program of intensified selection and breeding in limpograss was initiated by the author in 1976. The objectives of this program are better persistence under frequent cutting, improved IVOMD, and increased early spring production. In this selection program germplasm has been identified which is consistently above 70% IVOMD at five weeks growth. Other lines have produced 4.0-4.5 MT/ha of dry matter by April 20, 1978. Although limpograss is vegetatively propagated, crosses are being made in an attempt to incorporate high IVOMD, early growth, and persistence into a desirable forage type. Advanced lines are being evaluated for persistence under grazing.

In addition to these programs with perennial grasses, Dr. Gordon Prine has used recurrent selection for reseeding ability to develop a reseeding annual ryegrass, Lolium multiflorum L. for Florida and the lower South. This ryegrass has been named 'Florida reseeding' and is presently being increased in Oregon. A few commercial seed are expected to be available for the 1979 fall planting and adequate supplies for fall 1980. Florida reseeding shows superior reseeding over commercial cultivars if grazing is deferred so that seed can develop on pastures. Various annual and perennial clovers, big-flowered vetch and hop clover have reseeded satisfactorily with Florida reseeding ryegrass.

#### SELECTION AND BREEDING OF LEGUMES IN FLORIDA

By Albert E. Kretschmer, Jr.

Legume selection and breeding programs for Florida forage must satisfy a range of climatic zones from tropical to temperate.

Dr. L. S. Dunavin, ARC (Agricultural Research Center), Jay, is observing and selecting introductions primarily of birdsfoot trefoil (Lotus corniculatus), cicer milkvetch (Astragalus cicer) and flat pea (Lathyrus sylvestris). Major clover emphasis is on white, red, and sub.

At Gainesville, Dr. G. M. Prine, working with perennial peanuts has selected an Arachis glabrata, 'Florigraze', adapted to latitudes up to about 30°N and to well-drained soils. It produces from 4 to 6 tons of hay annually from 2 or 3 cuts. Florigraze competes well in perennial grass sods and survives under heavy grazing. Prine is also screening and selecting sub, arrowleaf, crimson, persian, red and alsike clovers; and vetches, hop clovers, and seradella.

Dr. C. E. Dean, clover breeder, is releasing a new, yet unnamed, white clover cultivar as soon as sufficient seeds are available. This variety has superior stolon persistence during the summer, resulting in earlier fall and winter utilization. Other work includes the identification and incorporation of genetic resistance to viruses.

Dr. E. S. Horner's alfalfa selection work led to the release of 'Florida 66'. At present his efforts have been directed to the selection of spotted alfalfa aphid resistance. An alfalfa population, selected for adaptation and persistence in the Gainesville environment for eight cycles, has been screened for aphid resistance for two cycles. The resistant population has performed well in variety trials and is being increased for release. Work will continue with this and two other recent releases for improved persistence and productivity.

Results of work at the ARC-Ft. Pierce has led to this year's release of 'Florida' carpon desmodium (Desmodium heterocarpon (L.)DC.), a long-lived, perennial, high seed-producing, tropical legume. It competes well with tropical grasses and persists under high grazing pressures.

At the ARC-Ft. Pierce, Dr. J. B. Brolmann, is working with the <u>Stylosanthes</u> genus including introductions of <u>S. scabra</u>, <u>S. guianensis</u>, <u>S. subsericea</u> and <u>S. fructicosa</u>. Screening of these species and natural stylo hybrids are being evaluated for persistence, early flowering, cold tolerance, and morphological characters. Characterization and evaluation of 60 native ecotypes of the perennial, S. hamata, are in progress.

Dr. A. E. Kretschmer, Jr. is evaluating about 900 tropical legume ecotypes including Macroptilium, Centrosema, Teramnus, Desmodium, Desmanthus, Calopogonium, and Aeschynomene. Particular emphasis will be in the evaluation of about 250 ecotypes of Aeschynomene for perenniation and tolerance to waterlogging.

#### GRAZING MANAGEMENT RESEARCH WITH IMPROVED FORAGES AT GAINESVILLE

## By W. R. Ocumpaugh

In addition to reporting on grazing research at Gainesville, the grazing research at Jay, Florida is to be covered.

Jay is in Northwest Florida in an area that is better adapted to growing row crops than most of Florida. Therefore, they have limited their grazing research to work on annual forage crops. In the past they have worked with summer annuals, but presently they are limiting their grazing research to winter annual crops. They plant winter pastures on land that is used to grow soybeans and other summer annual row crops. They do not maintain a herd of cattle on the station, but buy and sell feeder cattle to fit their needs. Their most recent work involves supplemental feeding of cattle on pasture at various rates, then taking some of these on into a feedlot situation for various lengths of time.

Grazing research on improved forages at Gainesville is more complex than at most research stations. We divide grazing research into at least two phases, as part of a multiphase forage evaluation scheme. The use of this scheme in a grass breeding program was reported to this group last year (Quesenberry et al. 1977).

One of these phases involves the use of the grazing animal only as a defoliation tool. Here we study the effects of the animal on plant responses, ie. botanical composition changes, survival and/or productivity.

Another of these grazing phases involves the more traditional method of studying the effects of plants (forage) on the animal responses.

We presently have only one experiment of this more traditional type where we are interested in animal performance. This experiment consists of using summer annual forages as supplemental creep grazing for nursing calves. One replication of the experiment is at the Pine Acres Research Unit south of Gainesville, the other replication is on the Beef Research Unit north of Gainesville.

The remainder of our grazing research effort is concentrated on studies of the effects of grazing animals on plant responses. All of this work is being conducted at the Beef Research Unit. This research is carried out using mini-sized pastures (0.05 to 0.10 ha each).

We have one experiment which contains 82 pastures to study the effect of grazing management in combination with other cultural treatments on a smutgrass-bahiagrass-white clover sward. Our main objective is to study the effects of these treatments on botanical composition changes of the sward, in hopes to learn how to manage pastures infested with smutgrass.

We have a number of grazing experiments where we have planted from 10 to 30 different breeding lines and/or plant introductions of forages within one pasture and grazed them at a set frequency using mob grazing techniques. In these experiments, we are mainly interested in survival and general vigor after one to three years of grazing. We also estimate relative yields of these

forages, usually with the aid of a simple disk-meter.

We have another variation of this where we introduce frequency of animal defoliation as a variable. In these experiments, we like to narrow the number of forages down to 10 or less. These are usually forages that were selected out of previous trials and constitute more advanced material in a breeding/selection program.

Still another type of experiment where we are looking at the effect of grazing animals on plants involves an experiment which has bahiagrass as the base grass. Here we have overseeded plots with 'Florida reseeding' ryegrass and 'Gulf' ryegrass and then planted subplots to 'Nolins' red clover, 'Nolins' white clover and an advanced breeding line of 'FS-5' white clover. This experiment contains 22 pastures, and we are imposing grazing management on these that we hope will help us learn more about how to manage the ryegrass so it will reseed and the clovers so they will live over the summer.

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#### FORAGE RESEARCH AT ONA

# By P. Mislevy

In the state of Florida there are about 34 million acres of total land area. Approximately one-third or 12 million acres is used for some form of pasture (native range, woodland and improved). About 4.5 million acres of this land is used for improved and temporary pasture or forage production. At the present time about 125,000 additional acres/year of native land is established to improved perennial or temporary forages via the vegetable route. Under this program land in native condition is used for 1-3 years for vegetable production, after which it is established into improved forages.

The Agricultural Research Center (ARC), Ona is located latitude 27°25' north, longitude 81°55' west. Climatically the weather is tropical with temperate intrusions in the winter season. These intrusions bring repeated frost periods having temperatures of 28°-34°F, with lower readings at less frequent intervals. Rainfall averages 56 inches annually with over 75% occurring from late May to mid October.

The Spodosols and associated flatwood soils found at the Ona research center occupy 8.5 million acres in Florida. These lands are well suited for forage production and also are used for citrus and vegetable production to a limited extent. Some 65% of the 2.8 million cattle found in Florida are located within the southern 2/3 of peninsular Florida. The research program at Ona, ARC is directed toward the need of the south central Florida cattle industry.

Due to the diverse rainfall, temperature and edaphic conditions between Gainesville and Ona 165 miles to the south, an intensive forage program is conducted at Ona. This program is conducted in cooperation with forage researchers in Gainesville and other research centers and provides much of the forage research information needed in the more tropical areas of the state. Forage research is conducted both at the Ona and Immokalee research center. The latter center is 100 miles south of the Ona ARC.

Research at Ona can be categorized in the following manner:

#### I. Winter Annuals

Each year some 10-15 entries each of small grains, ryegrasses, clovers, and alfalfas are tested to determine forage production, persistence, insect and disease problems and other agronomic characteristics. Entries are tested both at Ona and Immokalee research centers. They are seeded in November and harvested 4 to 6 times, terminating in May or June. Forage production can range from 3-5 T/A dry matter. Alfalfa and red clover varieties, which act as annuals, under Florida conditions are highest yielding, and produce forage over the longest time period of all winter annuals.

#### II. Summer Annual Grasses

Annually some 25-30 commercial corn hybrids, grain sorghums, and 10-15 entries each of forage sorghums, sudangrass x sorghum hybrids, and pearlmillets are tested for dry matter forage yield and/or grain yield, disease resistance, lodging and other agronomic characteristics. Most entries are tested at both the Ona and Immokalee locations. Entries are generally seeded in February and harvested in May, June and July, depending on species. Total seasonal dry matter yields may range from 6 T/A for pearlmillets to 18 T/A for forage sorghums. Dry matter forage yields for commercial corn hybrids generally range from 7 to 11 T/A in 100 days, with grain yields ranging from 120 to 180 bu/A shelled corn @ 15.5% moisture. Basic fertility programs vary from 150-100-200 to 250-100-200 lb/A N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O. Irrigation is applied on all summer annual grass studies.

#### III. Summer Annual Legumes

'American joint' vetch (Aeschynomene americana), 'Hairy indigo' (Indigofera hirsuta) and Alyce clover (Alysicarpus vaginalis) are the summer annual legumes grown in perennial grass sod and/or under cultivated conditions. Clipping studies are presently being conducted on American joint vetch and hairy indigo to monitor yield, quality and persistence. These legumes are seeded in June and grazed or harvested from late August through October. Fertility requirements are generally low with 0-30-60 lb/A N-P205-K20 as an adequate fertility program. Yields are also low, ranging from 1.0 to 2.0 T/A dry matter. Forage quality of these legumes when removed at a 12-20 inch height is generally quite good. All three species will withstand saturated soil conditions.

### IV. Perennial Forages

Perennial forage research generally follow the forage evaluation scheme described by Quesenberry et al (1977).

<u>Phase I</u>: Evaluation of plant introductions and breeder lines.--During this phase there are some 100-300 perennial forage entries evaluated as single plots, with sufficient space allowed for development of stoloniferous and/or rhizomatous plants. Entries expressing superior forage potential for a specific area are re-established in replicated plots. Forage potential is determined by forage yield, quality, persistence and vigor.

Phase II: Regional adaptation in small plot clipping trials.--All entries studied in this phase are in replicated plots. Presently various fertility and defoliation experiments are being conducted on Cynodon spp., Digitaria spp., Paspalum notatum, and Chloris gayana. Hydrocyanic acid is also being monitored at different fertility levels and physiological stages of growth in various Cynodon species.

Phase III: Forage response to grazing animals.--Forage research in this phase is conducted concurrently with phase two. When forages are selected to be eventually used for grazing, little would be gained by conducting phase two studies if the entry would not persist under grazing. At Ona various forms of phase III research are presently being conducted. This phase involves mob grazing of entries and grazing intensity studies.

The mob grazing technique involves confinement of a large number of

animals to small paddocks and forcing the animals to graze all entries to a uniformly close stubble height in one to two days. At Ona, the mob grazing technique is used as a method of screening 15-30 potential perennial forages. These forages are planted or seeded in individual plots 25 x 25 ft, surrounded by a 3 ft non-vegetative border. Pastures containing all of the grass entries are grazed at different frequencies. Rest periods of 2, 3, 4, 5 and 7 weeks are presently being studied. Prior to each grazing, treatments are sampled for dry matter yield, and quality. Forage persistence is also monitored throughout the growing season. Approximately forty yearling cattle are allowed to graze each 0.6 acre paddock. The purpose of this technique is to study the effect of the grazing animal on the forage entry.

The purpose of the grazing intensity experiment is to study the effect of stocking rate on forage yield, quality, utilization and animal performance. Three stocking rates SR (3 low), 4 (med) and 6 (high) cattle/A) were imposed on 3 stargrass entries (Cynodon spp.) and medium SR on 'Transvala digitgrass' (Digitaria decumbens) and 'Pensacola' bahiagrass (Paspalum notatum). Animal production was highest at the medium stocking rate averaging 600 lb/A/growing season over a two year period.

Phase IV: Animal response to forages.—The objective is to determine animal performance on potential perennial forages. Earlier, in phase III the effect of animal on plant performance was studied. The measurements in phase IV estimate animal gain per unit area, carrying capacity per unit area, voluntary intake, nutrient digestibility, forage quality prediction models, and forage yield in terms of feed units. Several of the variables described here were measured in the grazing intensity study above, indicating that certain variables in phase III and IV may over lap. Variables in phase IV are studied at Ona through year-long 5 acre pasture grazing experiments.

# V. Other Forage Research

Multicropping - Ona. -- The objective of this research is to produce two to three crops of high quality forage per year under water control. The present studies at Ona are divided into two parts, 1) demonstration and 2) research. The demonstration area contains 30 acres of tillable land surrounded by a 4' x 12' rim ditch and dike used for drainage. This multidisciplinary study will determine the physical and economic feasibility of growing several crops per year on the same land area and providing forage for animal feeding studies. Present multicropping research studies involve: cropping sequence studies (determining the proper forage crop sequence combination for central Florida), and corn-sorghum density study, and Aeschynomene clipping study. All studies are small plot clipping experiments in which superior treatments will be used in demonstration areas.

Multicropping - Immokalee. -- Many cattlemen are involved directly with vegetable production or indirectly involved through the lease of their land. The production of forage crops in rotation with tomatoes, peppers or cucurbits holds great potential, and the major reason is the use of residual fertility from the previous vegetable crop. At the Immokalee ARC a research program, is being carried out to study the production of forage crops which second as cover crops between vegetables. One study deals with the seeding of spring corn after fall tomatoes. Corn is drilled into the plastic mulch soon after the tomatoes are harvested. Another study is being carried out to select corn herbicides which are compatible with succeeding vegetable crops.

Sod-seeding. -- Research is presently being conducted with winter annual grasses and legumes and summer annual legumes at Ona and Immokalee. Experiments are designed to test seeding methods, species and herbicide treatments. Superior treatments are tested in demonstration plots throught central Florida.

Water Efficiency. -- This experiment is designed to determine the water-dry matter ratios of several perennial and annual forages during the cool-dry February through May period. In addition, water movement in a sandy soil is monitored after each irrigation or rainfall. Correlations between tensiometer readings at various soil depths and actual moisture are being calculated.

Phosphate reclamation. -- Due to the extensive phosphate mining in central Florida sand tailings spoil banks and slime areas (colloidal phosphate) are in need of reclamation. Therefore a series of studies were established to determine the optimum soil amendments required on sand tailings for good forage production. Grass and legume entries were established on the various soil treatments, to determine yield performance, drought tolerance, establishment and persistence of each entry. In addition experients were established to study the effect of plant species and fertilization on evapotranspiration as measured by the dehydration of a slime pond (colloidal phosphate).

Pasture herbicides. -- Research is presently being conducted on several weed species found in perennial subtropical pasture grasses. Smutgrass (Sporobolus poiretii) appears to be the most prevalent grassy weed. Dog fennel (Eupatorium capillifolium), thistle (Cirsium spp.), blackberry briers and horse nettle (Solanum carolinense) appear to be the most troublesome broadleaf weeds, in addition to prickly pear cactus (Opuntia spp.) in established perennial grass pastures.

Native rangeland. The native range represents a valuable resource for Florida cattlemen. Native pastures prior to 1960 were managed primarily for pineland threeawn (Aristida stricta), but emphasis has shifted to management for higher producing, more palatable bluestems (Andropogon spp.), indiangrass (Sorghastrum spp.), Panicum species, etc. Research is under-way at Ona to support this change in management direction. Measurement of the plant response to grazing and clipping, saw palmetto control, effect of fire and grazing, range rehabilitation, and chemical composition studies are a few of the research projects.

#### REFERENCE

Quesenberry, K. H., Rex L. Smith, S. C. Schank, and W. R. Ocumpaugh. 1977. Tropical Grass Breeding and Early Generation Testing with Grazing Animals. Proc. Southern Pasture and Forage Crop Improvement Conf. 34:100-103.

## FORAGE QUALITY EVALUATION AT THE UNIVERSITY OF FLORIDA

## By John E. Moore

The overall objectives of this research program are to help make improved forages and forage utilization systems available to Florida's ranchers and feeders, and to provide information which will assist in making forage—livestock management decisions. Specific objectives include the following:

- 1. Compare the quality of various forages in terms of animal performance, intake and digestibility.
- 2. To estimate the quality of forages from small research plots in terms of in vitro digestion and chemical composition.
- 3. To improve prediction methods by developing more accurate and rapid laboratory procedures for use in research and extension forage testing and evaluation.

#### EVALUATION WITH ANIMALS

The variety of soils and climates in Florida makes it necessary to conduct forage evaluation research with animals at several locations in north, central and south Florida, including Research Centers at Jay, Quincy, Ona, and Belle Glade, and in the Gainesville area at the Beef Research Unit, Purebred Beef Unit, Dairy Research Unit, Horse Research Unit, and Nutrition Laboratory. Grazing trials and feedlot trials are conducted with cattle, and intake and digestibility trials are conducted with cattle and sheep.

#### Permanent Grasses

Bahiagrasses, bermudagrasses, stargrasses, limpograsses (Hemarthria), digitgrasses, St. Augustinegrass, and paragrass are the primary permanent grasses under evaluation. There have been three releases in recent years: McCaleb stargrass, Slenderstem digitgrass, and Transvala digitgrass. Cattle grazing tropical grasses during the summer exhibit "summer slump", a period of low average daily gains due to low intake of forage. Studies of the effects of maturity have shown that after 8 weeks regrowth, voluntary TDN intake was below the maintenance requirement. Grain supplementation reduced intake of immature bermuda (substitutive) but had no effect on intake of mature bermuda (additive).

In the Everglades on organic soils, St. Augustinegrass was shown to be superior to bahiagrass and although paragrass was superior to both of the others, it was frost sensitive. Pastures and a high water table may be necessary to prevent subsidence of the soil and to maintain agricultural production in the Everglades. Pelleting of St. Augustinegrass and paragrass increased voluntary intake due to an increase in rate of passage.

## Temporary Grasses

Oats, ryegrass, wheat and triticale have been evaluated as temporary winter pastures, and sorghum and millet are being studied as pasture and as haylage in comparison to corn silage. With small grains, wheat was superior to rye and triticale, and supplemental feeding on pasture was not always profitable. Mixtures of forages including crimson clover lengthened the grazing season and increased beef production. Several ryegrass varieties including a tetraploid are being evaluated for intake and digestibility. The summer annuals were found to have a short growing season and in some cases, supplement was profitable. Corn was superior to sorghum as a silage crop and millet haylage was not as profitable as millet pasture.

## Legumes

Aeschynomene, alfalfa, clovers, perennial peanut, and <u>Desmodium</u> have been evaluated. Florida 66 alfalfa, a variety developed for Florida, and a new perennial peanut currently being evaluated, were shown to have high voluntary intake. The perennial peanut is being released for use in permanent pastures. Mature <u>Aeschynomene</u> haylage requires supplemental energy for growing steers because it has high fiber and low digestibility. <u>Desmodium heterocarpon</u> grows well in south Florida pastures and is currently being released.

## Sugarcane

Sugarcane whole plant, tops and bagasse are being evaluated as animal feed sources in the Everglades. Cane tops are suitable as a roughage source for finishing cattle and as a supplement to winter pasture for cows. The sugar content of whole cane depresses the digestibility of fiber and the rate of passage from the rumen. Sugarcane bagasse may be improved by sodium hydroxide treatment. Whole cane is being evaluated.

## Aquatic Plants

Water hyacinth and hydrilla, weeds which infest Florida's waterways, have been evaluated as feed sources for cattle. When incorporated in complete rations at the rate of 33% of the total organic matter, these plants were equal to bermudagrass as a source of nutrients. However, harvesting and storage problems limit the use of these materials at the present time.

#### ROUTINE LABORATORY EVALUATION

The forage evaluation laboratory in the Agronomy Department provides a service to forage researchers throughout the state. The major techniques are in vitro digestion and nitrogen analysis. The in vitro procedure involves two-stage organic matter digestion and has a capacity of 300 tubes per week. The nitrogen technique is automated and has a capacity of 200 determinations per day. Phosphorus is also determined by the automated procedure and cations are determined in the Soil Science Laboratory. The extension service provides a forage testing and evaluation program for farmers and ranchers in cooperation with the State Department of Agriculture and Consumer Services. Crude protein and crude fiber are used to estimate digestible protein, TDN and net energy.

The <u>in vitro</u> procedure is the most reliable predictor of forage quality now available, although there is a discrepancy in the <u>in vitro</u> and <u>in vivo</u> relationship with bahiagrass. Nevertheless, the technique has been useful in comparing introductions, breeders lines and management treatments. It was successful in predicting seasonal gains by steers grazing small grain pastures.

#### PREDICTION RESEARCH

Standard chemical analyses have been unacceptable as predictors of forage quality (intake of digestible organic matter) across a wide range of tropical grass species. Present research involves the development of rational mathematical models which describe digestion and passage, and the determination of forage characteristics which have cause-effect relationships with parameters in the model. Several forage characterization techniques including physical, anatomical and chemical methods are being evaluated. Infrared reflectance spectroscopy is being tested to determine if it has potential for making the numerous analyses necessary to improve acceptability of routine forage tests.

#### SUMMARY

Forage evaluation research is being conducted at nine units from north Florida to the Everglades. The wide range of climate and soil makes it necessary to evaluate a wide range of forages and non-conventional forage crops. A complete scope of analytical techniques is involved including animal performance trials on pasture and in feedlot, intake and digestibility trials, and routine in vitro and chemical analyses. Attempts are being made to improve the acceptability of predictions of forage quality using laboratory techniques.

## N<sub>2</sub>-FIXATION RESEARCH WITH TROPICAL GRASSES

By K. H. Quesenberry, R. L. Smith, S. C. Schank

The indication that significant levels of N<sub>2</sub>-fixation occur under tropical grass cover was suggested by nitrogen balance observations carried out by Dobereiner, 1961, 1966; Moore, 1963; and Joiyebo and Moore, 1963. Most of the world paid little attention to this research, until the energy crisis of the 1970's, when renewed interest in biological N<sub>2</sub> fixation arose because of the tremendous increases in the price of nitrogen fertilizers.

In Brazil in 1974, Drs. Rex L. Smith and S. C. Schank saw the potential of nitrogen-fixing associations between bacteria and tropical grass roots, particularly on 'Transvala' digitgrass. Dr. Rex Smith brought a culture of the suspected nitrogen fixing microbe, then called <u>Spirillum lipoferum</u>, to Florida and immediately began some exploratory experiments on inoculation of this bacteria onto the roots of several tropical grass species.

The results of these experiments raised sufficient interest at Florida in 1975 to stimulate the organization of a research team of plant breeders, physiologists, and microbiologists to study this associative system.

Results of the 1974 field inoculation experiment were verified in 1975, with more extensive experiments which showed that yield increases were possible at certain fertility levels on several of the tropical grasses. Data from the 1976 and 1977 inoculation experiments also showed similar trends, but a severe drought was experienced in early summer (1977) and few statistically significant yield increases were obtained from 1977 experiments. Since field results have been erratic, more controlled laboratory and greenhouse experiments have been conducted in an effort to better understand the system.

One greenhouse experiment with several genotypes of bermudagrass showed an apparent grass genotype-bacteria strain specificity. This result supported 2 year field testing of pearl millet genotypes which produced increased yields from inoculation of 'Gahia-3' but not from the inoculation of its parents. Additional experiments are underway to further study this possible genotype interaction.

We have utilized the acetylene reduction assay as a measure of potential N<sub>2</sub> fixation by grass-bacteria systems. Our research has shown that the rates are erratic and can be influenced by environmental conditions of the plants as well as altered conditions in the assay procedure. Higher levels of acetylene reduction were obtained in reduced oxygen environments. Acetylene reduction rates generally did not correlate with increased yields.

Dr. M. H. Gaskins, a plant physiologist on the N<sub>2</sub> research team, has shown that <u>Azospirillum brasilense</u> produces growth regulating compounds. He has suggested that the yield increases observed in field experiments may be due in part to these growth substances. Dr. Gaskins has also shown that the quantity of exudate from roots of small plants in sterile solution culture would not support high rates of N<sub>2</sub> fixation. These findings have not been tested on plants grown under field conditions, but genetically altered bacteria strains are being produced which should allow field testing of these findings.

Research is currently in progress to study environmental factors which may influence the establishment and continuation of an associative nitrogen fixing system. The effect of growing plants in a reduced 0, root environment with Azospirillum inoculum is being studied. Preliminary results from this study suggest that higher acetylene reduction rates are obtained in lower 0, 0ther studies are being planned to alter the photosynthate supply to the roots by shading.

#### SUMMARY

Inoculation of certain tropical grasses with Azospirillum brasilense can result in a significant yield increase. Most increases have been on the order of 15-20% over uninoculated controls. Response to inoculation is often erratic and unrepeatable, but there are some indications of a plant genotype-bacteria strain interaction. Although Azospirillum is a nitrogen fixing microbe, it also has been shown to produce growth regulating substances which may account for some of the observed yield responses. The effects of various environmental factors on the association are being studied.

[The references cited in this paper were not received for publication.--Publisher.]

# SYSTEMS FOR MAKING, HANDLING, STORING AND FEEDING LARGE HAY PACKAGES

## By B. L. Bledsoe

Scarcity of labor for timely harvest — and hay quality losses from weather damage or from excessive mechanical manipulation — are haymaking problems which have plagued farmers for years. In the early 1970's mechanical large-package haymaking developed as one method for more timely harvest with fewer personnel. Systems of machines were introduced to allow one person working alone to conduct all operations necessary to harvest, transport, store, retrieve and feed hay. However, shapes and sizes of the large packages created problems for inside storage. Quality deterioration from outside storage caused concern — especially for high-value legume hays commonly used for dairy operations in the humid Southeast. Consequently, experiments at various locations have attempted to determine the extent of losses caused by large package haymaking, storage and feeding methods, and to define new methods for reducing the losses.

This report describes results from experiments with large-package having systems at The University of Tennessee and compares them with findings of other research efforts in the Southeast and in some other regions of the nation.

The Tennessee experiments were accomplished cooperatively by the Departments of Agricultural Engineering, Plant and Soil Science, and Animal Science. The experiments were at Ames Plantation, Grand Junction, at the Dairy Experiment Station, Lewisburg, and on two private farms, one in East Tennessee and one in Middle Tennessee. The studies emphasized machines requiring low initial investment such that results would be applicable to small farms (less than 100 ha).

## CUTTING, CONDITIONING, CURING BEFORE PACKAGING

To reduce chances for weather damage, hay should be cut and dried to the moisture content required for packaging as quickly as possible. Conditioning — crimping, crushing, or abrading plant stems and leaves — will hasten drying (1,6,7,10) of both grass and legume hay. For small stemmed grasses, like the bermudagrasses, Hellwig (7) concluded that the fluffy windrows left by conditioning machines — altering the way the hay lies on the ground — had a greater influence on improved drying rates than did the physical alteration of the grass stems and leaves by the conditioning rolls. In criteria for an improved hay conditioner for the temperate, humid climate of the British Isles, Klinner (10) stressed the formation of a low density swath or windrow that resists settling and is deposited on a uniform stubble capable of supporting the cut crop to optimize air circulation.

The horizontal rotary-head tedder (Figure 1) is widely used in humid areas of Europe as a hay conditioning device. Barrington and Bruhn (1) found that alfalfa-brome hay tedded with this machine immediately after cutting dried faster than untreated swaths but not so fast as hay crushed by conditioning



Figure 1.--Horizontal rotary-head tedder used in swath-drying studies.

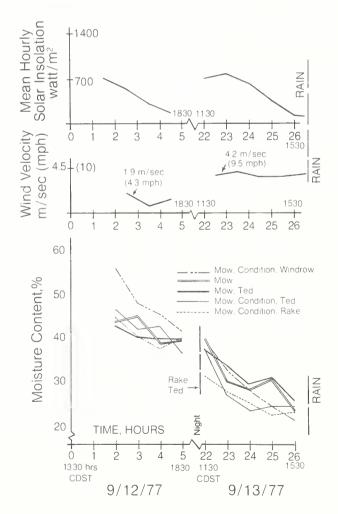


Figure 2.--Drying curves for five swath-drying treatments, Ames Plantation, 1977.

rolls and left in a swath to dry. Hellwig (7) compared a cylindrical reel tedder with a crushing roll conditioner and found no significant difference in drying rates for Coastal bermudagrass. He also noted that bermudagrass stems were not adequately crushed by one pass through the rolls. This observation led him and others to experiments with a tandem roll mower-crusher (8). Two passes through the crusher rolls increased the drying rate of Coastal bermudagrass hay about 40 percent above that conditioned with only one pass. The hay in vitro dry matter digestibility was improved significantly by the extra pass through the conditioner rolls. However, unrecoverable forage yield (due to breaking of grass stems into small pieces that were lost in the stubble) was 14.3% with the tandem mower-crusher, but only 10.3% with the conventional mower-crusher; this increased loss was considered excessive.

In the Tennessee experiments, a horizontal rotary-head tedder was used to form partially dried swaths of conditioned hay into windrows for completion of drying. The fluffy windrows were expected to dry faster than the denser windrows formed with a side-delivery rake. Three years of data show that the fluffy windrows (cross-sectional area from 1.2 to 1.8 times that of raked windrows) did not dry faster than raked windrows unless wind velocity was above 6.5 km/hr (4 MPH) and relative humidity was below 50%. Drying rates of the five treatments compared in 1977 using Midland bermudagrass at Ames Plantation are shown in Fig. 2. Wind velocity was great enough on September 13 to give air circulation for faster drying in the tedded windrows, but high humidity preceding the rainfall on that day offset the drying advantage afforded by the wind.

Note the one-pass mow-condition-windrow treatment was the slowest to dry on September 12. That treatment would have been slower on the following day also, except for the more rapid uptake of moisture from the high humidity air by the other four treatments. The one-pass mow-condition-windrow treatment is popular with some farmers because of the economy of machine use (fewer trips over the field), but it is the slowest way to prepare hay for packaging, as has been reported by researchers in Wisconsin (1) and Kansas (6) and elsewhere.

In humid areas of Europe, the tedder is used to scatter and fluff hay immediately behind the cutter. Tedding is then repeated one or two times to get a homogeneous dry matter content within 2 days if possible (4). Using the tedder in this manner will increase hay particles lost in the stubble, but the loss is considered a minor penalty for the increased drying rate. Tedding without windrowing will be evaluated in future Tennessee experiments. Another promising treatment to be evaluated is to partially dry conditioned hay in a thin, wide swath, then to invert the swath without bunching or windrowing to expose the bottom of the swath to sunlight for completion of drying.

#### FORMING HAY INTO LARGE PACKAGES

A wide choice of hay packaging machines is available. High density roll balers, producing packages of 360 kg (800 lb) and 680 kg (1500 lb) with mean wet weight density of 150 kg/m $^3$  (9.4 lb/ft $^3$ ) are the most popular machine types used by smaller-farm operators in Tennessee. Low density roll balers making 540 kg (1200 lb) packages of 80 kg/m $^3$  (5 lb/ft $^3$ ) density and 1000 kg (1.1 ton) compressed stack machines are other machines in use. Many larger-farm operators prefer large compressed stack machines, making stacks containing 2700 kg (3 tons) or 5400 kg (6 tons) of hay. These large stacking units provide efficiency in packaging and handling large amounts of hay but have purchase prices 1.6 to 2.5 times those of the smaller stacker.

TABLE 1. Mean packaging capacities for three machines used with Midland bermudagrass and Kobe lespedeza at different moisture levels

Machine	Нау Туре	Moisture Content	Packaging Capacity*
Vermeer-605C Hawk-Bilt-480 Stakhand-10	Bermudagrass Bermudagrass Bermudagrass	18.5 & 24.4 18.5 & 24.4 18.5 & 24.4	1000 kg/hr ton/hr 11.28 <sup>a</sup> 12.43 <sup>a</sup> 22.73 <sup>b</sup> 25.06 <sup>b</sup> 6.30 <sup>c</sup> 6.94 <sup>c</sup>
A11	Bermudagrass	24.4	$15.21_{b}^{a} 16.77_{b}^{a}$ $11.67^{b} 12.86^{b}$
A11	Bermudagrass	18.5	
Vermeer-605C	Lespedeza	12.3 & 21.4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Hawk Bilt-480	Lespedeza	12.3 & 21.4	
Stakhand-10	Lespedeza	12.3 & 21.4	
A11	Lespedeza	21.4	$13.66_{b}^{a}$ $15.06_{b}^{a}$ $5.76_{b}^{a}$ $6.35_{b}^{a}$
A11	Lespedeza	12.3	
Vermeer-605C Hawk Bilt-480 Stakhand-10	Both Both Both	12.3, 18.5, 21.4 & 24.4	$8.96_{b}^{a}$ $9.88_{b}^{a}$ $20.60_{b}^{b}$ $22.71_{b}^{b}$ $5.17_{c}^{c}$ $5.70_{c}^{c}$
A11	Both	21.4 & 24.4	$14.45_{b}^{a}$ $15.93_{b}^{a}$ $8.71_{b}^{a}$ $9.60_{b}^{a}$
A11	Both	12.3 & 18.5	
A11	Bermudagrass	12.3, 18.5,	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
A11	Lespedeza	21.4 & 24.4	

\*Means within each category having similar superscripts are not significantly different at  $\alpha$  = 0.05.

Data from Robertson et al., 1976 (21)

Tennessee studies compared a high-density roll baler producing twine-wrapped rolls of 680 kg (1500 lb) (Vermeer 605C), a low-density roll baler producing 540 kg (1200 lb) rolls not wrapped with twine (Hawk Bilt 480) and a low-density compressed stack machine producing stacks of 1000 kg (1.1 ton) (Hesston StakHand 10). Mean packaging capacities observed for these machines during one series of experiments are listed in Table 1 (21). Windrows for the packaging capacity experiments contained hay from 4.3 m (14 ft)-widths of a Midland bermudagrass field yielding 850 kg/ha (2.3 ton/ac) and from a Kobe lespedeza field yielding 650 kg/ha (1.7 ton/ac). Operating conditions were ideal and allowed the skilled operators to attain near maximum capacity for the machines. The time required to wrap the high-density rolls with twine reduced packaging

capacity as compared to the non-twine-wrapping low-density roll baler. However, the high density rolls could be moved from the field immediately, whereas the low-density rolls and stacks needed to remain in the field where discharged from the machines for a minimum of 24 hours to allow the herbage to settle and coalesce into packages stable enough for handling.

Bale chamber losses were not evaluated in these tests. However, other investigators have shown that large-package machines have losses greater than conventional balers because the hay is more severely manipulated for a longer period of time (15). Losses of large package machines also are highly dependent on moisture content of the hay, especially leguminous hays subject to much leaf shattering when dry. Losses in conventional balers vary from 2 to 5% regardless of windrow size or hay moisture content, but bale chamber losses in large-package machines vary, depending on moisture content, from 5 to 15% with alfalfa hay. For round balers, heavy windrows of hay at maximum moisture content for safe storage are recommended to reduce bale chamber losses. This requirement is just opposite that for a dense roll with maximum water-shedding ability. A denser roll develops from use of a small windrow giving more layers or wraps per roll. A dense roll sheds water better than a looser one but prevents air circulation necessary for curing hay baled at moisture content above 20 to 25% (21).

#### MOVING HAY PACKAGES TO AND FROM STORAGE

Various means by which one person can handle and transport large hay packages up to  $1000~\rm kg$  are listed in Table 2. The handling capacity ( $1000~\rm kg/hr$ ) depends on transport distance and safe travel speed as well as on time required for loading and unloading. Twine-wrapped rolls can be handled and transported more expeditiously than loose rolls or stacks. When packages are moved from storage to the feeding area, the part of the package that contacted the ground is usually partially decayed and tends to fall off if packages are handled roughly. Multiple-roll bale movers which rotate bales in handling — and discharge them with a different surface in contact with the ground than existed in storage — tend to result in a significant loss of hay (20).

Renoll et al. recommended using a self-loading 2-wheel trailer mover towed by a pickup truck for moving single rolls distances greater then 1.6 km (1.0 mi). However, an on-farm case study in Middle Tennessee (14) showed that the tractor -mounted single-roll mover had greater handling capacity with less cost for distances under 3.2 km (2 mi) than the trailer-type mover. Evidently topography and field and road conditions have a strong influence on handling and transport capacity (Table 3).

#### STORAGE OF HAY PACKAGES

A number of experiments has confirmed that inside storage of hay packages with moisture content below 20% results in good hay quality preservation (5,15, 21). However, well-formed stacks with no depressions in the top surface, and rolls of grass hay can be stored outside on sunny, well-drained surfaces with only small dry matter and quality losses. The packages should be spaced at least 0.3 m (1 ft) apart for air circulation to remove moisture accumulations after rain. Dry matter loss is dependent on type of hay, climatic conditions, package density and moisture content of the hay when packaged.

In Missouri (5) alfalfa rolled into large bales at 36% moisture content and stored outside lost 30% dry matter but only 19% when the rolls were stored

TABLE 2. Methods for handling and transporting hay packages

Description	Approximate Handling Capacity <sup>†</sup>	Approximate Price	
	1000 kg/hr	\$	
Trailer type mover for single roll bale; manually operated winch	1.8	600	
Tractor-mounted (3-point hitch) mover for single roll bale	4	250	
Tractor-mounted (front-end loader attachment) mover for single bale	4	250	
Tractor-mounted (3-point hitch) mover for 1-ton stacks	6.5	900	
PTO-driven roll bale pickup and trans- port trailer (3 to 4 roll capacity)	6.5-8	4500	

Average transport distance of 1.3 km (0.8 mi) calculated from data in references (3) and (20).

TABLE 3. Capacity of low-cost, one-roll transport methods

		Dicc H	1. D.:
Method	0.8 km <sup>†</sup>	Different Hau	3.2 km <sup>†</sup>
		_ 1000 kg/hr	
3-point hitch or front end loader attachment for tractor	6.5	2.2	1.6
Truck towed 2-wheel trailer mover	2.7	1.8	1.5

 $<sup>^{\</sup>dagger}$ Data from Merritt, 1978 (14); 4-wheel drive pickup truck used.  $^{f}$ Data from Renoll et al, 1977 (20).

1 km = 0.62 mi

1000 kg = 1.1 ton

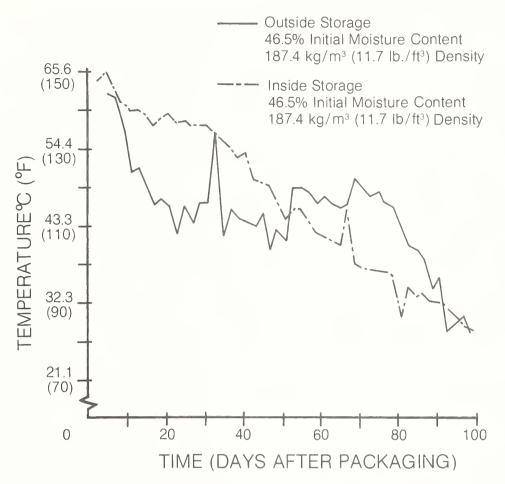


Figure 3.--Change of internal temperature with time of high-initial-moisture Midland bermudagrass hay in high-density rolls.

inside for 6 months. Peak measured internal temperature after the 7th day of storage for rolls stored inside on end with 2 m (6.6 ft) center-to-center spacing was 61 C (142 F) and temperatures above 38 C (100 F) persisted for 80 days. Rolls stored outside had peak internal temperature of 67 C (153 F), but temperatures above 38 C (100 F) persisted for only 74 days. Digestible protein content of the hay decreased rapidly during the first 6 days of storage, when bale temperatures — indicative of microbial activity — were highest, then stabilized. The digestible protein decrease for hay in rolls stored inside was 3% (from 14 to 11%), and in rolls stored outside 4% (from 14 to 10%). In contrast to the high moisture alfalfa, fescue hay baled at 24.5% moisture had dry matter losses of 3.3% in rolls stored inside and 13.9% in rolls stored outside.

In Nebraska (23) experiments indicated the nutritive value of alfalfa hay could be maintained in compressed stacks ( $74 \text{ kg/m}^3$  ( $4.6 \text{ lb/ft}^3$ ) dry matter density) when packaged at moisture contents up to 40%. In Montana (12) alfalfa hay was formed into 2700 kg (3 ton) and 5400 kg (6 ton) low-density compressed stacks at moisture contents ranging from 18 to 53% and into 500 kg (0.6 ton) high-density rolls at moisture contents ranging from 14 to 39%. The packages were stored for 3 months and monitored for hay quality changes. The results indicated storage losses were less at higher moisture but lower density.

An Iowa experiment (15) with mixed alfalfa-clover hay determined that dry matter loss increased with an increase in initial moisture content of large hay packages, but packages stored outside had dry matter losses of less than 5% when initial moisture content was no greater than 40%. Dry matter loss increased markedly when package maximum temperature exceeded 49 C (120 F). After a six-month storage, large rolls of hay with plastic caps over the upper 2/3 of the surface contained the best quality hay.

In the more humid climate of Alabama, Renoll et al. (18) found that low-density compressed stacks had dry matter losses of 14%, but a large part of the loss was due to decay of hay in contact with the ground during storage. Similar results occurred with storage of high-density roll bales (19).

In an Indiana experiment (17) outside storage of hay packages on a 15 cm (6 in) layer of crushed stone resulted in 10% less storage loss than when the packages were stored on sod.

Initial experiments in Tennessee with large package haymaking (1972-73) indicated that quality of high moisture (22-30%) Midland bermudagrass hay was preserved better in low-density than in high-density packages (9). Outside storage of high-moisture packages led to better quality than inside storage where rolls were closely spaced in the barn. Bale internal temperatures of high-density rolls packaged at 46.5% moisture content are compared for inside and outside storage in Fig. 3. Air circulation around packages stored outside resulted in more rapid initial cooling.

In later experiments (1973-74) large packages of Midland bermudagrass and Kobe lespedeza (21) were compared. At equal initial moisture contents, the lespedeza hay packages had greater internal temperatures than the grass hay — indicative of greater microbial activity. Effect of initial moisture content on package internal temperature for lespedeza hay is shown in Fig. 4. Those lespedeza packages made from hay of 23% initial moisture content had internal

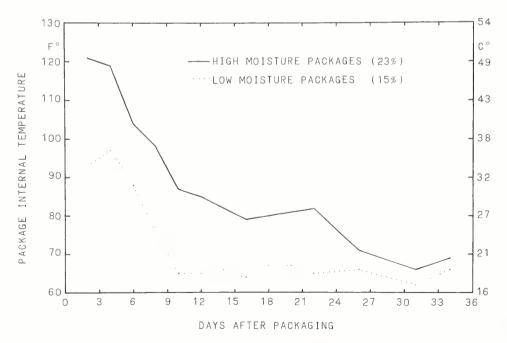


Figure 4.--Curing temperatures for high-and low-moisture lespedeza hays at several package densities.

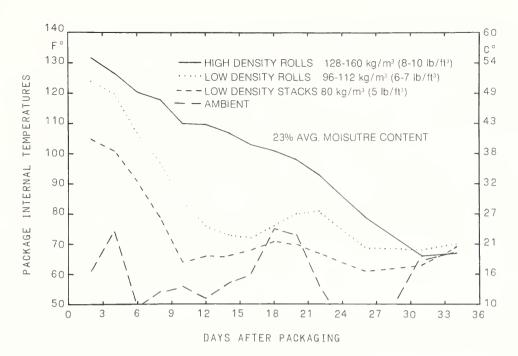


Figure 5.--Mean curing and ambient air temperatures for three package types of of bermudagrass and lespedeza high-moisture hays.

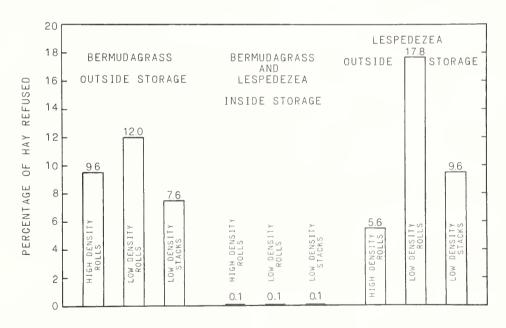


Figure 6.--Percentage of hay refused from three package types when fed in sliding gate feeders.

temperatures above 49 C (120 F) for 3 days, whereas internal temperatures for packages made from hay with 15% moisture content never exceeded 38 C (100 F).

A comparison of the mean curing temperatures of bermudagrass and lespedeza hays at 23% moisture content shows the effect of package density on curing rate (Fig. 5). Internal temperatures of the low-density packages responded to marked changes in ambient air temperature; high-density package internal temperatures did not. This difference resulted from air flow through the more permeable low-density hay.

A waterproof thatch was not formed readily on the outer surface of the lespedeza packages, unlike in grass hay packages. Thus lespedeza hay was preserved better in high-density packages than in low-density ones. Quality was preserved better with inside storage of lespedeza packages than with outside storage (Fig. 6).

Three storage methods — inside, outside on automobile tires, outside on ground — were compared for alfalfa-orchardgrass hay in high-density rolls at the Dairy Experiment Station (2). Mean initial moisture content of the hay was 11.5% and package dry matter density was  $120~{\rm kg/m^3}$  (7.6  $1{\rm b/ft^3}$ ). Consumption of hay by lactating Jersey cows for each storage method is summarized in Table 4. Note the advantage in dry matter preservation resulting from placing rolls on automobile tires for outside storage. Weathered hay for the rolls stored outside extended 20 cm (8 in) radially inward from the surface. Peak package internal temperature for rolls stored outside was 52 C (125 F) and 54 C (129 F) in rolls stored inside.

## FEEDING LARGE HAY PACKAGES

Large hay packages offered unrestricted to cattle in the open field resulted in excessive losses — up to 45% of package wet weight (18). The use

TABLE 4. Dry matter losses in high-density rolls of alfalfaorchard grass hay

Dry Matter	Stored	Stored Outside			
Dry Hatter	Inside	On Tires	On Ground		
		%			
Losses in Storage	3.4	11.9	16.0		
Losses from Refusal and Waste	3.7	14.1	17.4		
Apparent Consumption	92.9 <sup>a</sup> *	74.0 <sup>b</sup>	66.6 <sup>b</sup> **		

<sup>\*</sup>Difference in values followed by the same superscript are not significant at  $\alpha$  = 0.05.

Data from Baxter, <u>et al.</u>, 1978 (2).

<sup>\*\*</sup>Difference between rolls stored on tires and on ground was significant at  $\alpha$  = 0.10.

TABLE 5. Feeding losses from large rolls of sorghum-sudangrass hay fed with and without circular panels

Treatment †	Dry Matter Feeding Losses				
Treatment	By Refusal From Trampling		Total		
		%			
No panel, roll axis horizontal	24.3	3.6	27.9		
Panel, roll axis horizontal	6.5	2.9	9.4		
Panel, roll axis vertical	5.4	2.7	8.1		

 $<sup>^{\</sup>dagger}500$  kg (1100 lb) rolls of 34.5% moisture content when baled; dry matter density of rolls when baled was 84.7 kg/m $^3$  (5.3 lb/ft $^3$ ); dry matter loss during 6-month storage was 14.2%.

of feeding panels, bunks, or racks for feeding large hay packages can reduce these losses. Parson  $\underline{\text{et}}$   $\underline{\text{al}}$ . (17) noted that unrestricted feeding of large grass hay packages to beef cows required 10.9 to 12.9 kg (24 to 28 lb) of dry matter/cow day. With racks the hay requirement dropped to 9.2 kg (20 lb) dry matter/cow day.

The type of feeding equipment required depends on climate. Where feeding areas are dry (or frozen) fields, panels that cattle can push toward the hay package as they eat are required for large stacks. For roll bales, either circular or rectangular fixed-geometry panels that encircle the bale are satisfactory for dry feeding areas. Slanted-bar access openings keep cattle from backing away from the racks while eating and pulling hay ouside where it can be trampled. Dry matter losses when feeding sorghum-sudangrass hay in large rolls to non-lactating dairy cows on a private farm in East Tennessee (22) with feeding-panels were one-third those measured when feeding rolls without panels (Table 5).

In muddy feeding areas and during rainy weather, trampling losses with floored and covered feed bunks were kept below 5% during feeding trials conducted during the rainy month of December, 1974 at Ames Plantation (21).

#### FUTURE DEVELOPMENT IN HAYMAKING

Large hay packages can be made, handled and fed efficiently by one person. However, with hay of low moisture content, excessive losses occur from manipulation of the hay by the packaging machine pickup and bale chamber mechanisms. To overcome this problem, two approaches have been proposed by investigators and machinery manufacturers: (1) make large rectangular balers with hay manipulation mechanisms similar to those of small rectangular balers, or (2) make large rolls and stacks from high-moisture hay (35 to 45% wet basis) to prevent leaf loss during manipulation. To preserve the high moisture hay, addition of chemical preservatives (11,13), ventilation of the package, or drying with heated air have geen suggested.

#### SUMMARY

Large package haymaking allows one person, with suitable equipment, to carry out packaging, handling, storing, and feeding. With such a system, accelerated drying of the hay in the swath before packaging is still important to avoid weather damage to the hay.

Storage losses associated with use of large package hay depend on climate, type of hay, package density, and moisture content of the hay when packaged. In humid climates, such as in Tennessee, grass hays can be packaged into low-density rolls or stacks at moisture contents in the range of 20 to 30% (wet basis) with low dry matter and quality losses when stored outside. Leguminous hays required high-density rolls or stacks at moisture content below 20% for quality preservation when stored outside. Storing packages on crushed stone or on old automobile tires to avoid contact with the soil will reduce decay and dry matter losses in the package.

Transporting large hay packages with minimal losses is best done with machines that do not rotate the package and that deposit it in the same position relative to the ground after transport as it was before the move. For small-farm operators, roll-bale mover attachments for tractor 3-point hitch or front-end loader are more economical for haul distances up to 3.2 km (2 mi) than self-loading trailer movers. The trailer movers, however, are more economical for distances greater than 3.2 km (2 mi).

To avoid losses from leaf shattering during the prolonged and aggressive manipulations characteristic of present large hay-packaging machines, manufacturers and investigators are developing large rectangular balers and means for either drying or using chemicals to reduce bacterial action in large packages of high-moisture hay.

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## EVALUATING FORAGE CHARACTERISTICS USING A DYNAMIC MODEL OF FIBER DISAPPEARANCE IN THE RUMINANT

By D.R. Mertens and L.O. Ely

#### INTRODUCTION

The objective of many forage evaluation programs in animal science and agronomy is to assess forage quality from data on chemical and physical characteristics of the feed. Although many factors have been suggested and evaluated as determinants or indicators of forage quality, most have been discarded or found to be of limited use when used as the sole index of forage nutritive value. This suggests that accurate assessment of forage quality must include the interactions of the animal and its microorganisms with the chemical, morphological and physical properties of forages and the end-products resulting from their utilization.

Since it may not be feasible to measure and evaluate all relevant factors and interactions involved in forage quality in a single experiment, it was concluded that modeling and simulation can offer an excellent opportunity to delineate the role of animal and plant characteristics in forage fiber digestion. A model of forage fiber digestion could provide information about digestibility, intake, end-product production and nutrient utilization. Digestibility can be easily related to the digestive mechanism because it is a function of the kinetics of digestion and passage (3,29,46). Intake of forages is related to fiber digestion because it is limited by the rate of disappearance of material from the digestive tract (11,16,17,29,39,45). Recent research suggests that rate of digestion or passage influences the proportion of end-products (volatile fatty acids) produced during fermentation in the rumen (23).

A dynamic, mathematical model was developed to include the kinetics of passage, particle size reduction and digestion to describe the disappearance of forage fiber from the digestive tract of ruminants. The objectives of model development were: (1) to determine if current theories of digestion, passage and particle size reduction could be described adequately by mathematical equations and combined into a model to estimate fiber disappearance from the digestive tract; (2) to identify aspects of ruminant digestion and forage characterization where current concepts or data are inadequate; and (3) to test hypotheses regarding plant and animal factors influencing forage quality. The model will be described and used to assess quantitatively the factors affecting the digestion of alfalfa and Coastal bermudagrass.

## MODEL DEVELOPMENT AND PARAMETER ESTIMATION

The model was developed by deriving a series of differential equations that described theoretical relationships concerning digestion, passage and particle size reduction. Each submodel (digestion, passage or particle size reduction) was developed to represent, as simply as possible, current concepts

of animal physiology and plant characteristics; yet to include the features of applicability, accomodation, manageability, and output comparability suggested by Baldwin  $\underline{\text{et}}$   $\underline{\text{al}}$ . (5). The model was implemented on the computer using the Continuous System Modeling Program (CSMP). The model was verified to assure the accuracy of mathematical formulation and computer programming in implementing the model and was validated by comparing model output to research observations.

## Passage Submodel

The basic submodel is a sequential compartmental system proposed by Blaxter et al. (9) and Brandt and Thacker (10). This model assumes that  $R^{k7}>I^{k8}>F$ , where F is material excreted in the feces and R and I are digestive compartments in the animal. Coombe and Kay (15) and Grovum and Williams (20, 21) suggest that the I compartment is the large intestine and k8 represents the rate of passage of material from the large intestine to the feces, leaving compartment R to represent the rumen and k7 to represent the rate of passage (or escape) of material from the rumen to the large intestine. The passage of material through the omasum, abomasum and small intestine is assumed to be a linear process that has no first-order kinetic properties. Data of several researchers (9,15,20,21,22,27,34) were combined and interpolated to obtain ruminal escape and large intestine rates of passage.

#### Particle Size Reduction Submodel

It is evident that when long forages are fed particle size reduction must occur before fiber particles escape from the rumen. Ulyatt  $\underline{\text{et}}$   $\underline{\text{al}}$ . (41), Troelsen and Campbell (40) and Van Soest (44) showed that average particle size decreases as material passes from feed to rumen contents to abomasal contents or feces. Although it may be possible to describe fiber particles in the rumen by a normal, or log-normal, distribution, observation of stratification of matter in the rumen suggests that the rumen is not a homogenous mass of fiber particles (13). In addition, the observation that large particles do not pass out of the rumen suggests that the rumen must contain at least two pools of fiber particles—one pool of large particles that must be reduced in size before passage and another pool of small particles that can escape the rumen.

The basic rate of passage submodel was modified by dividing the rumen compartment (R) into large (RL) medium (RM) and small (RS) particle subcompartments. Three subcompartments were chosen based upon the research of Matis (27) which indicated that fecal marker excretion was most accurately predicted when three subcompartments were used. In addition, Ulyatt et al. (41) and Evans et al. (18) reported similar trimodal distributions of particle sizes in rumen contents. The inclusion of a medium size particle subcompartment also permits the passage of some particles that are larger than those in small particle pool. Van Soest (44) reported that mean fecal particle size increases with increasing intake in dairy cows. Incorporation of this concept in a two subcompartment system would require the possibility that very large particles escape the rumen.

Particle size reduction between compartments was assumed to follow first order kinetics  $RL^{k_4}RM^{k_5}RS$ . The model was further modified to allow feed entering the rumen to enter RL, RM, or RS depending upon the proportion of

the feed that was of large, medium or small size. In addition, some medium particle (RM) material was allowed to escape from the rumen but the rate was very slow. The data of Evans et al. (18) were used to determine rate of particle size reduction and the relative distribution of the three subcompartments in the rumen.

## Digestion Submodel

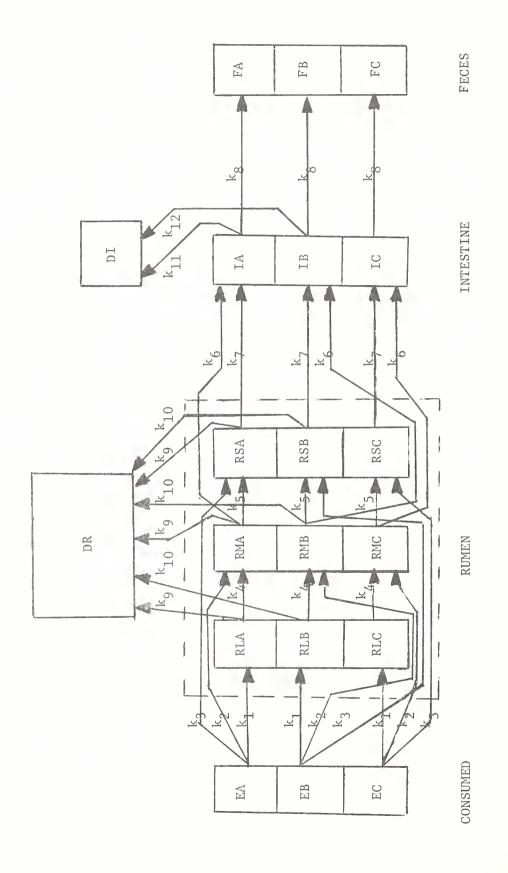
The basic model for rate of digestion was first described by Waldo (29), who postulated that forage fiber could be divided into digestible and indigestible fractions and that rate of digestion of the digestible fraction might exhibit first-order kinetic behavior. Although the concept of an indigestible fiber fraction is not accepted by all researchers, several in vitro and in vivo experiments indicate that digestion reaches a maximum that does not equal 100% (6,29,36,45,49) and that probably at least the lignin component of fiber is not completely digested in ruminants.

Smith et al. (35,36) used 72 hr in vitro indigestibility to estimate the indigestible fraction and determined rates of neutral detergent fiber (NDF) digestion in several forages. Mertens and Van Soest (30) observed that some digestion occurs beyond 72 hours; thus the 72 in vitro estimate will overestimate indigestibility. Although the mean time that material remains in the rumen is 40-60 hours, approximately 10-25% of forage fiber remains in the rumen for more than 70 hours. When the maximum extent of digestion is determined and used to define the indigestible fraction (C), overall digestion is more accurately predicted by assuming that the digestible fraction contains fast-digesting (A) and slow-digesting (B) compartments. Dividing fiber into three fractions is supported by the recent research of Akin et al. (1,2), which suggests that forage plants have three morphological tissue types which have different rates of disappearance. They observed that mesophyll and phloem tissue were fast-digesting, bundle sheaths and epidermal cells were slow-digesting, while lignified vascular bundles and sclerenchyma tissues were relatively indigestible.

Rates of digestion and proportion of fiber in fast-digesting, slow-digesting and indigestible fractions were obtained from Mertens (29). Since the effect of particle size upon rate of digestion has not been clearly defined, it was assumed that digestion rate would be the same for all particle sizes in the rumen. Although Ulyatt  $\underline{\text{et}}$   $\underline{\text{al}}$ . (42) have suggested that celluloytic activity in the large intestine is equal to or greater than that observed in the rumen, the digestion rate of fiber in the large intestine was assumed to be 90% of that of the rumen because fiber reaching the large intestine would be more refractory to digestion than that present in the rumen.

## Complete Model

The complete model of fiber disappearance from the digestive tract of ruminants is given in figure 1. Rates of particle size reduction, passage and digestion have been integrated in a complete model of fiber disappearance. The model was described by 20 differential equations and implemented on the computer using CSMP. The output from CSMP gives the pool sizes (amount of fiber in each subcompartment) and fluxes (amount of material passing between compartments per unit of time) of fiber as it passes through the digestive system. Starting with initial estimates of the pool sizes and given rates of



Block diagram of the model of fiber dynamics through the entire digestive tract of the C = indiruminant. Legend: A = fast-digesting fraction, B = slow-digesting fraction, C = ingestible fraction, D = digested, E = consumed, F = feces, I = intestines, L = long, M = medium, R = rumen, and S = small Figure 1.

disappearance, the model soon reaches steady state conditions for each set of forage kinetic characteristics used. At steady state the amount of material eaten (E) per unit of time equals the amount digested and appearing in the feces (D+F) per unit of time. The pool sizes at steady state then represent the average amount of material that would be found in the various segments of the animal's digestive tract.

The model was evaluated by simulating the effects of feeding a 500 kg steer at the forage intake level of 2% of body weight per day. Input was provided as 24 hourly feedings of equal size during each simulated day. Steady state conditions for fiber content, digestion and excretion pool sizes were usually reached within 1 to 2 simulated days after rates of digestion or proportion of indigestible neutral detergent fiber were changed. Time required for adaptation of the simulated steer depended upon the magnitude of the change in diet. Changing complete diets required 2 to 6 simulated days before steady state conditions were attained, corresponding to times typically needed by animals to adapt to similar changes in rations. All data presented in this paper were obtained from steady state conditions.

The complete model was used to simulate NDF digestibility (NDF Dig), percentage of total digestion that occurred in the rumen and amounts of NDF in the various model pools. Rates of passage and particle size reduction shown in figure 1 were held constant as follows: Particle size distribution; k=.85 or .00, k2=.05 or .10, and k3=.10 or .90, for long or pelleted forage, respectively: Particle size reduction and passage;  $k_4=.07$ ,  $k_5=.14$ ,  $k_6=.006$ ,  $k_7=.035$ , and  $k_{o}=.08$ . Dry matter digestibility (DMD) was calculated from the summative equation of Goering and Van Soest (19) :DMD=.98(100-NDF)+(NDF Dig) (NDF)-12.9. Predicted maximum dry matter intake was calculated based upon the assumption that the maximum rumen fill capacity of a 500 kg steer would be 7.92 kg of NDF (29). By simulating total rumen NDF from all pools at various intakes of dry matter the relationship between total rumen NDF and intake was derived and solved for 7.92 kg of NDF to obtain the predicted maximum dry matter intake. Maximum digestible dry matter intake was derived by multiplying the predicted maximum dry matter intake by the dry matter digestibility coefficient thereby obtaining an estimate of the maximum amount of available energy that would be consumed by the animal.

Since rigorous validation of the model was impossible because no data are available where all, or even a majority, of the variables were measured, the complete model was validated by comparing model output to several sources of published research observations. The model was accepted as valid because it could accurately predict fiber digestibility, fiber and lignin contents of various gut segments, lignin turnover times, rate of passage and dry matter intake (3,4,7,8,11,12,24,25,26,28,31,33,37,38,43,47,48). Acceptance of the model was supported by the observation that the original estimates of differential equation coefficients, which were obtained from a variety of in vitro and in vivo experiments, required very little fine tuning to obtain output comparable with experimental observations.

## FACTORS AFFECTING FORAGE QUALITY

Two forages, alfalfa and Coastal bermudagrass, were selected for simulation to assess the factors affecting forage quality. The composition and kinetic values presented in table 1 represent the average of 39 alfalfa and nine Coastal bermudagrass samples (29). The crude protein and neutral detergent

TABLE 1. CHEMICAL COMPOSITION AND KINETIC CHARACTERISTICS OF TYPICAL ALFALFA AND COASTAL BERMUDAGRASS FORAGES USED IN SIMULATION EXPERIMENTS

CHARACTERISTIC	ALFALFA	COASTAL BERMUDAGRASS
Crude protein (% dry matter)	18.5	16.6
Neutral detergent fiber (% dry matter)	46.6	70.0
Acid detergent fiber (% dry matter)	36.3	34.7
Permanganate lignin (% dry matter)	9.7	5.4
Fast-digesting fraction ( $\%$ NDF $^a$ )	42.4	51.7
Fast-digesting rate $(hr^{-1})$	.1012	.0919
Slow-digesting fraction (% NFDa)	9.6	15.2
Slow-digesting rate $(hr^{-1})$	.0190	.0224
Indigestible fraction(% NFDa)	48.0	33.1

<sup>&</sup>lt;sup>a</sup>Percentage of the neutral detergent fiber.

fiber (NDF) contents of these forages indicate they are high quality (14). The primary differences between alfalfa and Coastal bermudagrass are in NDF and lignin contents and the proportion of NDF that is slow-digestiing. Alfalfa contains less NDF than Coastal bermudagrass but the NDF is more lignified and contains a smaller slow-digesting fraction.

## Effect of Physical Form of the Forage

The effect of processing forages to alter physical form by grinding and pelleting is shown in table 2. The model predicts that digestibility of both alfalfa and Coastal bermudagrass are decreased by grinding and pelleting. This effect observed by many researchers (3,7,8,24,25,26,31,33,38,45,48), has been attributed to reduced retention time in the rumen, i.e., increased rate of passage out of the rumen. This hypothesis agrees with the model which predicts that NDF and lignin turnover or retention times in the rumen are decreased by pelleting. Dry matter digestibility was decreased to a greater extent by pelleting Coastal bermudagrass then by pelleting alfalfa. This result could be due to differences in NDF content between the forages. Alfalfa contains less NDF than Coastal bermudagrass and the use of the summative equation of Goering and Van Soest (19) to predict DMD permits changes only in the fiber portion of the forage to alter DMD while the non-NDF fraction is held constant at 98% digestibility. However, the difference between alfalfa and Coastal bermudagrass in digestibility depression due to processing could be due to differences in quality between the forages (31).

Processing forage also alters the site of fiber digestion. The model predicts that approximately 93% of the digestion of long forage fiber occurs in the rumen. This agrees with reported ruminal digestions of NDF or cellulose fed as long forage that range from 85 to 100% alfalfa (24,26,38) and 81 to 94% for grasses (8,26,43). The model predicts that 84% of the pelleted forage fiber is digested in the rumen which compares favorably with reported values of 60 to 94% for alfalfa (24,26,38) and 75 to 89% for grasses (8,26,43).

As shown in table 2, model simulation indicates that pelleting these forages results in less fiber fill in the rumen which permits greater maximum dry matter intake if rumen volume were the factor limiting intake. Simulation results also suggest that the amount and proportion of fiber in the intestines increases when forages are pelleted, agreeing with the observations of Hodgson (25) and 0'Dell et al. (33) which suggest that capacity of the lower digestive tract does not limit the intake of long forage.

Increased intake appears to be the major factor in improved performance observed when roughages are ground and pelleted (7). Although it is generally accepted that less response is expected from pelleting a high quality forage than a low quality one, simultion results suggest that alfalfa has a larger intake response (1.41 units) than Coastal bermudagrass (1.02). There is little data supporting the generally accepted concept that pelleting high quality forages obtains less response than low quality forages (7) although many of the small responses from pelleting high quality forages can be explained by intake limitations imposed by energy demands of the animal. The data of Minson and Milford (31) and Weston and Hogan (48) support the results of model simulation that intake of high quality forages is improved to a greater extent than low quality forages. Weston and Hogan (48) observed that intake was increased 1.16 units for alfalfa and .68 units for wheaten hay when the forages were pelleted.

TABLE 2. EFFECT OF PHYSICAL FORM OF FORAGES UPON THE DIGESTION, FIBER TURNOVER TIMES, FIBER CONTENTS OF THE DIGESTIVE TRACT AND MAXIMUM DRY MATTER INTAKE DETERMINED BY SIMULATION

Variable <sup>a</sup>		LFALFA Pelleted	COASTAL BERMUDAGRASS Long Pelleted	
NDF <sup>b</sup> Digestibility (%)	46.3	41.8	58.9	52.8
NDF <sup>b</sup> Digestion Occurring in Rumen (%)	93.7	84.0	93.0	83.2
Dry Matter Digestibility (%)	61.0 58.9		57.7	53.5
Rumen NDF <sup>b</sup> Content (kg)		3.70	7.01	4.82
Rumen NDF <sup>b</sup> Turnover Time (hr)	28.4	19.1	24.0	16.5
Rumen Lignin Turnover Time (hr)	45.8	29.4	45.8	29.4
Intestinal NDF <sup>b</sup> Content (kg)		1.42	1.50	1.74
Maximum Dry Matter Intake (% BWC)	2.87	4.28	2.26	3.28
Max. Dig. Dry Matter Intake (% Bwc)	1.75	2.52	1.30	1.75

 $<sup>^{\</sup>mathrm{a}}$ All values except intake were simulated for a 500 kg steer consuming 10 kg of forage dry matter daily

<sup>&</sup>lt;sup>b</sup>Neutral detergent fiber

 $<sup>^{\</sup>rm C}{\rm Percentage}$  of body weight consumed daily by a 500 kg steer.

## Effect of the Indigestible Fraction of Forages

Understanding the plant characteristics that influence digestibility and intake is important in developing new methods for evaluating forages and improving their utilization. Lignin is generally accepted as the primary antiquality factor that inhibits the utilization of fibrous carbohydrates in forages (32). Recently, Smith et al. (36) and Mertens (29) observed that lignin content of the plant is most highly associated with the indigestible fraction of NDF. If this relationship is causal, the effect of lignin upon animal performance can be demonstrated by changes in the indigestible fraction of NDF.

Table 3 presents the simulation results when the indigestible fraction of NDF was increased or decreased 8.1% for alfalfa and 21.1% for Coastal bermudagrass. As expected, increasing the indigestible fraction decreased digestibility. It also increased the contents of NDF in the rumen and intestines and resulted in decreased maximum dry matter intake. A 16.2% decrease in the indigestibility fraction of alfalfa produced a 5.6% increase in digestibility and 9.8% increase in intake resulting in a 15.4% increase in maximum digestible dry matter intake. A 42.3% decrease in the indigestible fraction of Coastal bermudagrass obtained a 18.2% increase in digestibility and a 25.2% increase in intake which resulted in a 43.8% increase in maximum digestible dry matter intake. Thus, a one percent decrease in the indigestible fraction results in a 1.0% increase in maximum digestible dry matter intake.

## Effect of Fiber Digestion Rate

Crampton (17) and others (16,39,45) suggested that rate of digestion is important in assessing forage quality, especially voluntary intake. It was postulated that increased disappearance of fiber from the digestive tract by more rapid rate of digestion would free space for additional intake. The effect of changing rates of digestion were simulated (table 4). Rates of fast-and slow-digesting fractions were increased or decreased 15% for both alfalfa and Coastal bermudagrass. A 30% increase in digestion rates resulted in a 1.5 or 3.6% increase in dry matter digestibility, a 4.5 or 7.5% increase in intake resulting in a 6.3 or 10.8% increase in maximum digestible dry matter intake for alfalfa and Coastal bermudagrass, respectively. Thus, a one percent increase in digestion rates results in a 0.6% increase in maximum digestible dry matter intake.

#### Effect of Rumen Fiber Turnover Time

Retention time or turnover time of rumen contents has been associated with changes in both digestibility (3,46) and intake (11,39). Comparisons of NDF and lignin turnover times in tables 2,3 and 4 provide information about factors affecting turnover and variables influenced by changes in turnover. Since the rate constants for passage and particle size reduction were held constant for long hay, the turnover time of lignin, which can disappear only by passage, is constant (45.8 hrs). However, the turnover time of NDF fed as long forage varies from 21.1 to 29.8 hrs. Although the turnover time of a digestible component such as NDF represents the total effect of disappearance from the rumen (39) it provides little insight into the mechanism affecting disappearance because digestion, passage and particle size reduction are con-

TABLE 3. EFFECT OF THE PROPORTION OF THE NEUTRAL DETERGENT FIBER THAT IS INDIGESTIBLE UPON THE DIGESTION, FIBER TURNOVER TIME, FIBER CONTENTS OF THE DIGESTIVE TRACT AND MAXIMUM DRY MATTER INTAKE DETERMINED BY SIMULATION

Variablea	ALFALFA		COA BERMUD	STAL AGRASS
Indigestible fraction (% $\mathrm{NDF}^{b}$ )	44.1	51.9	26.1	40.1
NDF <sup>b</sup> Digestibility (%)	50.0	42.7	66.4	51.4
${ m NDF}^{ m b}$ Occurring in Rumen (%)	93.8	93.6	93.8	93.5
Dry Matter Digestibility (%)	62.7	59.3	63.0	52.5
Rumen NDF <sup>b</sup> Content (kg)	5.24	5.79	6.14	7.87
Rumen NDF <sup>b</sup> Turnover Time (hr)	26.9	29.8	21.1	27.0
Rumen Lignin Turnover Time (hr)	45.8	45.8	45.8	45.8
Intestinal NDF <sup>b</sup> Content (kg)	1.22	1.39	1.23	1.77
Maximum Dry Matter Intake (% B $\hat{\mathbb{W}}^{C}$ )	3.02	2.74	2.58	2.01
Max. Dig. Dry Matter Intake (% BW <sup>c</sup> )	1.89	1.62	1.63	1.06

 $<sup>^{\</sup>mathrm{a}}$ All values except intake were simulated for a 500 kg steer consuming 10 kg of forage dry matter daily

<sup>&</sup>lt;sup>b</sup>Neutral detergent fiber

 $<sup>^{\</sup>mathrm{c}}\mathrm{Percentage}$  of body weight consumed daily by a 500 kg steer

TABLE 4. EFFECT OF RATE OF DIGESTION OF NEUTRAL DETERGENT FIBER UPON THE DIGESTION, FIBER TURNOVER TIME, FIBER CONTENTS OF THE DIGESTIVE TRACT AND MAXIMUM DRY MATTER INTAKE DETERMINED BY SIMULATION

Variable <sup>a</sup>		ALFALFA		STAL AGRASS	
$\mathrm{NDF}^\mathrm{b}$ Digestion Rate (hr <sup>-1</sup> )	.1163	.0860	.1056	.0782	
NDF <sup>b</sup> Digestibility (%)	47.2	45.2	60.1	57.2	
${\tt NDF}^b$ Digestion Occuring in Rumen(%)	94.2	92.9	93.6	92.2	
Dry Matter Digestibility (%)		60.5	58.6	56.5	
Rumen NDF <sup>b</sup> Content (kg)	5.40	5.66	6.77	7.30	
Rumen NDF <sup>b</sup> Turnover Time (hr)	27.8	29.1	23.1	24.9	
Rumen Lignin Turnover Time (hr)	45.8	45.8	45.8	45.8	
Intestinal ${ m NDF}^{ m b}$ Content (kg)	1.28	1.33	1.46	1.56	
Maximum Dry Matter Intake (% BWC)	2.93	2.80	2.34	2.17	
Max. Dig Dry Matter Intake (% BWC)	1.80	1.69	1.37	1.23	

 $<sup>^{\</sup>mathrm{a}}$ All values except intake were simulated for a 500 kg steer consuming 10 kg of forage dry matter daily

<sup>&</sup>lt;sup>b</sup>Neutral detergent fiber

cPercentage of body weight consumed daily by a 500 kg steer

founded. Comparison of values in tables 2, 3 and 4 suggest that physical form, indigestibility and digestion rate alter NDF turnover time.

Since the turnover of digestible materials is difficult to interpret, the turnover of indigestible materials (such as lignin) is more useful in obtaining insight into the mechanisms affecting digestibility and intake of forages. However, the turnover of indigestible markers, especially lignin, probably represent the upper limit of turnover time for components of digesta. Data presented in table 2 can be used to assess the effect of turnover time upon digestion and intake since pelleting reduced lignin turnover time compared to long forage. A 43.6% reduction in lignin turnover time yielded a 3.4 or 7.3% decrease in digestion and 49.1 or 45.1% increase in intake for alfalfa or Coastal bermudagrass, respectively. This resulted in an increase in maximum digestible dry matter intake of 44.0% for alfalfa and 34.6% for Coastal bermudagrass. Thus, a one percent decrease in turnover time results in a 0.9% increase in maximum digestible dry matter intake.

#### SUMMARY

A dynamic model of fiber disappearance from the digestive tract of ruminants was developed based upon acceptable and defensible concepts of fiber digestion and passage kinetics, and coefficients obtained from available literature. The model has applicability, manageability and comparability to experimental observations. Although the model can simulate the effects of some forage and animal characteristics upon digestion, it should be realized that it is only the initial component of an overall rumen function model. Specific limitations of the present model include the aggregation of microbial interactions with fiber and fiber characteristics into the digestion rate constants and the assumption that fiber digestion is not limited by factors other than fiber characteristics.

Development and use of the model suggested the need for additional information in several aspects of ruminant digestive function. More research is needed concerning particle size reduction, including: (1) particle size distributions in feed, digestive tract and feces; (2) changes in particle size in the rumen associated with rumination and chewing; and (3) description of particle size reduction as a factor influencing rate of passage. Information is also needed in describing digestion such as the effect of: (1) particle size; (2) microbial interaction; and (3) chemical, morphological or physical plant characteristics upon digestion rate. Additional research is also needed to obtain usable rate of passage coefficients under a variety of animal states and dietary situations.

Although improvement of the model of fiber disappearance in ruminants will need to continue as new information is accumulated, it can be used in its present form to assess some factors that influence forage quality. Simulation of the model provides controlled evaluation of the beneficial effect of grinding and pelleting forages. Analysis of plant and animal characteristics that influence digestion and intake suggests that the proportion of the NDF in the indigestible fraction and rate of passage influence the maximum intake of digestible dry matter more than rate of digestion.

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#### CATTLE CYCLES - RESEARCH RESPONSE

#### By Marvin E. Riewe

That the cattle cycle exists was confirmed again in the mid 70's. It is still a fact of life. The basic phenomenon has not been altered but extenuating circumstances magnified the effect of the cattle cycle on the economic well being of the cattle producer in the 70's.

The cattle cycle has been described as a 10-year poker game. This description seems apt. The survivors of a cattle cycle remain and get older. One fourth of the cows in Texas in 1974 were owned by people over 65 years of age; three fourths were owned by people over 45 years of age (5). It seems reasonable to assume that the recent shake out has concentrated the ownership of cows among older people even more. Many of the losers leave the game. The turnover rate of people in the cattle business is high. As prices improve on the upswing in the next cycle, new players are attracted. Many people with few cows try to play the game. To illustrate, 60% of the cow-calf producers in Texas owned only 20% of the cows in 1974.

The question I would like to examine today is: Has the forage-beef cattle research and extension education of the past 25 years largely fed the idiosyncrasies of the cattle cycle rather than help producers develop a strategy for coping with it?

We could dismiss the question by taking the position that science and research is amoral. We could argue that we as research and extension workers have no control over what the producer does. The producer decides how he uses new technology. If he keeps too many cows, produces more calves than the market can absorb at an acceptable price, he does so on his own initiative. Neither research nor extension education is in anyway responsible. To argue this position is to say, however, that our work has little influence on the producer. Yet, our operational, tactical and strategic research (4) are specifically designed, if not explicitly stated, to do just that.

#### REVIEW

Let us begin by reviewing the scenario of the last cattle cycle, 1965-1975. Beef calf production was increased from 12,412,000 head to 17,835,000 head in the 13 southern states represented here today, a 44% increase. Outside of the South, the remainder of the U. S. increased calf production 32% during this same period (6). Much of the research done by people in this group here today made possible the feed resources that were required to sustain this increase in calf numbers. The increase in cow numbers probably was not justified by the more limited increase in feed resources. Some of you have questioned this too (1).

Table 1. Effect of stocking rate on calf weaning weight, cow gain on pasture and length of winter feeding, 1976-77 and 1977-78, Angleton, Texas

Stocking rate expressed as cow-calf pair per acre	expressed as Ave. wean wt. cow-calf pair per calf, lbs.		Cow wt. gain, grazing season*, lbs. <sup>a</sup>	No. days winter feeding <sup>b</sup>
.5	600	522	236	67
.7	584	528	212	74
.9	538	483	142	110
1.2	501	475	117	114

<sup>&</sup>lt;sup>a</sup>All weights taken after 15-hr. overnight shrink.

When prices drop, pounds calf weaned per cow must increase to pay costs. For example, non-pasture cost of \$125 per cow annually can be paid for by 167 pounds per cow with calves selling at \$75 per hundredweight. At selling price of \$50 per hundredweight, 250 pounds calf weight per cow is required and 500 pounds calf weight per cow is required with a selling price of \$25 per hundredweight.

Research has demonstrated that weaning weights can be increased by improved quality of the pasture forage including, in particular, growing a palatable legume in the pasture mix, systematic crossbreeding to capitalize on hybrid vigor, use of bulls of the larger beef breeds and use of dams with potential for increased milk production for the nursing calf. While definitive weights are difficult to obtain, market weights that are available do not indicate significant increases in weaning weights, industrywide, in the past 25 years. These potential gains in calf weaning weight are apparently being wiped out by some other factor.

Increasing grazing pressure on pastures is one factor that reduces calf weaning weights. The effect of increased stocking rate (grazing pressure) on calf and cow gains is shown with data from a grazing study at Angleton, Texas involving four stocking rates, each replicated three time on a common bermudagrass, dallisgrass, La. S-1 white clover pasture fertilized with one hundred pounds of triple superphosphate per acre annually (Table 1). Forage availability was continuously monitored but the data is not presented here. Thus, it seems likely that the potential for increasing weaning weights through the use of such things as clover in pastures and crossbreeding was being wiped out by increasing the grazing pressure on pastures.

In addition, the trend, nationally, in calving percentage was downward (2) as cow numbers increased. Grazing pressure was affecting the cow. Since calf prices were improving during the upswing in the cattle cycle, a feasible

bFull feed sorghum silage (FS-lA) supplemented with urea.

<sup>\*</sup>Grazing season on pasture - late March to December or January depending on stocking rate.

method of relieving some pressure on the lactating cow was to wean the calves at an earlier age, lighter in weight. Dollar return was still acceptable to many producers.

It is at this point that the subordination of planned research to the idiosyncrasies of the cattle cycle are most apparent. As the effect of grazing pressure exerted upon the brood cow became apparent in the late 60's and early 70's, research was initiated in such subject matter areas as management of light, early weaned calves (grain was cheap) and reproductive physiology.

Prices rose dramatically during the first half of 1973, and then, just as dramatically, dropped the last half of 1973. The decline in prices continued through 1974 into early 1975. A freeze on beef prices, an embargo on oil, and high-price grains aggravated the situation, but these were not the causative agents. Excessive number of cows producing calves was the cause. Now, weaning weights were too light to spare the cow man of heavy losses.

#### WARNING SIGNALS

The National Cattlemen's Association has offered seven recognizable danger signals to warn of trouble ahead in a cattle cycle (3). Three indicators come from the size of the cattle herd - (a) the annual growth rate of the total cattle inventory exceeds 2%, (b) the annual growth rate of the cow herd exceeds 2% and (c) the number of heifers in the current calf crop saved for replacement exceeds 21% of the number of cows in the national herd. Slaughter rate indicators of trouble are (a) less than 35% of the January 1 inventory are slaughtered during the year, (b) annual female slaughter as a % of steer slaughter is less than 80%, (c) annual slaughter in numbers is less than 80% of the previous year's calf crop and (d) actual number of cattle slaughtered is 2 million head per year less than that required to maintain a stable inventory.

Historically, when three or more signals are "flashing red", some adjustment is likely. Six of these signals were "flashing red" in 1952 (those who remember will recall a major adjustment), and five were "flashing red" in 1963. The adjustments made in the mid 1960's in cattle numbers and price were straining but not necessarily severe. And that apparently lulled some, even economists, to sleep. Then again in 1972, five signals were "flashing red" and all seven were "flashing red" in 1973.

The problem is that the warning signals start "flashing red" when prices are high. No one wants to believe them. Perhaps this is what is meant by "blinded by greed." On the other hand, it is difficult to fault the producer when one reads in the Southern Cooperative Series Bulletin 186 (publication date, March 1974) "With beef prices at the 1973 level, beef is a more profitable enterprise (than) in 1968 and based on projected conditions is expected to remain in a profitable position for several years."

When the bubble burst, the cow-calf producers were weaning calves too light. Even when the market was signaling the producer not to send any more light calves to market, most did anyway. Many either could not or would not wean heavier. Presumably, they could not. The seriousness of the situation is shown in an analysis of saleable receipts June, 1975 through May, 1976 for one

Southeast Texas livestock market using the Packers and Stockyards Administration "model" (second week of each month) (2). The market was clearly signaling that the light calves were not wanted, yet 60% of the calves weighed under 400 pounds (Table 2). (It is assumed that only a few calves are in the 551 to 800 pound group and that these are more than offset by light cows in the 400-550 pound group). There has been little improvement in weight during the year just ended, June, 1977 through May, 1978 (Table 3).

If our research is not to be subordinate to the peculiarities of the cattle cycle, then the priority for our research and extension education effort would seem to be to provide the Southern producer the technology that allows him to develop a system of management for marketing heavy calves; say, a minimum of 550 to 600 pounds. This is necessary to reduce risks to producers incurred during the liquidation phase of the cattle cycle. To reduce the risks associated with the liquidation phase means that producers will resist certain temptations to maximize profits during periods of peak prices and excessive risk.

#### PROBLEMS TO OVERCOME

As people involved in forage-beef cattle research and extension education, we have some in-house problems to overcome to make our efforts more useful to the producer.

First, we must understand the industry and the producers we serve. The technology we develop must be acceptable to the user. If in the user's view, he must incur considerable risk in adopting the new technology, he will likely not adopt it. "True risk", given adequate data, can be estimated. But, "true risk", as far as the producer is concerned, does not count. Rather it is the risk as the user of technology perceives it that matters. One function of extension education might be to reconcile the producer's perceived risk with "true risk."

Having said this, I am left with a paradox. It seems difficult to reconcile the apparent willingness of many producers to accept the excessive risk associated with increasing numbers and yet not accept the lesser risk associated with managing the cow herd and pasture in a manner necessary for heavier weaning weights and reduced losses in the liquidation phase of the cycle. Perhaps it is that for most producers, probably 90% or more, cattle are not a primary source of income. Perhaps, the cattle business, for many producers, is even more like a poker game than we care to admit.

A second problem is false notions we harbor that increasing the carrying capacity of a pasture is of itself a worthy goal and that maximizing live—weight gain per acre also maximizes profit per acre. The non-pasture costs of maintaining an animal are paid for by production or gain per animal. In this, production or liveweight gain per acre does not count. We must become more sophisticated in our economics. This kind of thinking does indeed subordinate our work to the eccentricities of the cattle cycle. Risks in the cattle cycle are reduced by paying strict attention to the production or gain per animal with the view of getting the job done with the fewest animals possible. Geting the job done is balancing the risks involved with appropriate opportunities for profit.

Table 2. Composition of saleable receipts at one Southeast Texas livestock market, June, 1975 through May, 1976

		San	Average	
Class, weight-lbs.		Number	Percent	\$/cwt
Baby calves,	150 & less	479	1.5	17.15
Calves,	151 - 250	2,713	8.4	20.45
Calves,	251 - 400	12,404	38.3	24.25
Cows/Calves,	401 - 550	8,847	27.3	27.61
Cows/Calves,	551 - 800	4,534	14.0	22.29
Cows,	801 & up	3,175	9.8	21.11
Cow-calf pairs		70	• 2	22.96
Bulls		176	•5	26.43
Total in sample		32,398	100.0	25.12

Table 3. Composition of saleable receipts at one Southeast Texas livestock market, June, 1977 through May, 1978

Class, weight-lbs.		Sa	Average	
		Number	Percent	\$/cwt
Baby calves,	150 & less	233	• 7	25.57
Calves,	151 - 250	2,143	6.7	40.16
Calves,	251 - 400	11,065	34.7	38.71
Cows/Calves,	401 - 550	8,793	27.6	37.73
Cows/Calves,	551 - 800	5,305	16.6	29.86
Cows,	801 & up	3,984	12.5	26.92
Cow-calf pairs		89	.3	36.06
Bulls		268	.9	33.97
Total in sample		31,880	100.0	33.63

Another notion needs to be re-examined. "Minimal cost of beef comes from a 1,000 pound animal. In order to market the calf, then we must carry the calf on to about 1,000 pounds slaughter weight." I am aware of the classical studies which show that with the calf continuing to grow at a creditable rate and taking into account the maintenance requirement of the cow, the energy required to produce a pound of beef is minimal at slaughter weights of about 1,000 pounds. The problem is that the source of the energy is not taken into account. With the feed resources commonly available in the South, we can, without great difficulty, wean calves weighing in excess of 500 pounds. Programs heavily dependent upon warm season perennial grasses grown with a compatible clover have been designed which will produce calves weaning at weights of 600 pounds or heavier. Yet calves grazing these same pastures post-

weaning will not gain at a generally acceptable level unless they first endure a period of undernutrition to provide opportunity for compensatory growth. We need to examine the question of what is an optimal slaughter weight given the resources we have available, or are likely to have available, in the South.

A third problem of some concern is parochialism. By parochialism, I mean aspiring to develop production programs where we do not and likely will not have a competitive advantage. To illustrate, we are not likely to produce Choice grade slaughter beef in large numbers in the South unless Southwestern and Midwestern feeders first fail because of high grain prices. To attempt to capitalize on their failure would still leave us vulnerable with respect to competing for such inputs as nitrogen fertilizer. We would be much less vulnerable if we develop those areas where we have a competitive advantage such as cow-calf programs and then extend this to the production of slaughter animals grading less than Choice or Good.

#### RESEARCH NEEDED

What kind of research and extension education effort is required to develop strategy for coping with cattle cycles? Cow-calf programs are fundamental to the beef cattle industry in the South. Heavier weaning weights are extremely important in providing some degree of economic stability for our producers. This, then, suggests the following areas of priority research and extension education efforts.

A major effort is required to extend the use of palatable legumes in Southern pastures. The question regarding the need for legumes in Southern pastures should be resolved in most minds by now. Legume yield and persistence in pastures should be increased. Learning to effectively manage and utilize legumes in pastures is paramount.

The improvement of quality of warm season perennial grasses through breeding and/or management without sacrificing ease of establishment, persistence, cold tolerance, disease and drouth resistance is priority research.

Increasing the genetic potential for gain in the Southern cow herd is a prerequisite to coping with the cattle cycle. Research and extension education in this area must continue.

Each of you, I am sure, can suggest other areas of priority research. The point is that those things that make it possible to have more cattle without improving production or gain per animal tend to feed the idiosyncrasies of the cattle cycle. On the other hand, those things that improve production or gain per animal allow for development of a strategy for coping with the cattle cycle.

To have a stable beef cattle industry in the South, our producers must achieve economic maturity. The charge to research and extension education is to help our producers reach that maturity.

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#### GRAZING SUBTROPICAL PASTURES - COMPONENTS AND SYSTEMS

### By Elver M. Hodges

One of the first sources of improved pasture in south-central Florida was common bermudagrass that grew in garden and farm areas in response to cultivation and increased fertility. Planned development of better grazing for cattle centered around carpetgrass and common bahiagrass. Low input and low productivity values were associated with both varieties. Improved pasture fertilization for the 1940 decade consisted of 500 pounds per acre of 6-6-6, once annually, with a ton of dolomitic or calcic limestone added on a once-inseveral-years basis. The latter half of the 1940's saw extensive plantings of Pangola digitgrass and Pensacola bahiagrass along with increases in fertilization rates. Legumes on the flatwoods lands were limited to white clover, Hubam sweet clover, and a little black medic; some alvceclover was grown on soils with better drainage and higher fertility. These cool-season legumes were erratic in production but yielded dramatic increases in cattle gain per acre when all factors were favorable. It was demonstrated in the early 1950's that irrigation could be used to make white clover a reasonably reliable crop when managed intensively. Yield relationships between ordinary grass pastures and a highly successful clover-grass pasture ranged from less than 100 pounds per acre on carpetgrass and 300 pounds on Pangola to 800 pounds on irrigated white clover-grass. The need for consistent, high level management plus the water requirement placed a continuing limit on the acreage of white clover. During the era of moderate fertilizer costs it was observed that Pangola digitgrass responded strongly to increased rates of fertilization. Nitrogen levels of 100, 200 and 300 pounds per acre annually, applied in split applications, produced warm season per acre animal gains of 300, 468, and 568 pounds respectively.

Annual ryegrass (Lolium multiflorum) has been planted in lawns in peninsular Florida for many years. Its value for pasture was limited by rust damage, low soil fertility and dry weather. The development of rust-resistant varieties and a better understanding of plant food needs made ryegrass a useful possibility for winter and spring grazing. Sorghum-sudan hybrid forages have had value on better drained areas but ordinary flatwoods sites are too susceptible to extremes of drought and wetness.

Two annual warm season legumes, Hairy indigo ( $\underline{\text{Indigofera}}$   $\underline{\text{hirsuta}}$ ) and Aeschynomene ( $\underline{\text{Aeschynomene}}$   $\underline{\text{americana}}$ ), became available during the 1950's and were grown in combination with various perennial grasses. Hairy indigo proved to be less palatable than Aeschynomene and more emphasis has been placed on the latter variety.

TABLE 1.--Hay consumption, weaning rate and weight, and calf production per acre on eight forage and supplement systems. ARC, Ona, 5 years  $\frac{1}{2}$ 

System	Hay per cow lbs. annual	Weaning rate %	Weaning weight lbs.	Calf production lbs/acre
Grass	485	67	481	213
Grass + molasses	331	77	483	244
Grass + Hubam	706	68	467	213
Grass + Aeschynomene A	573	82	474	224
Grass + Aeschynomene B	639	75	434	205
Grass + whiteclover	529	69	483	221
Grass + ryegrass	154	72	507	218
Grass + ryegrass + sorghum	154	82	501	255
+ whiteclover				

<sup>1/</sup> E. M. Hodges, F. M. Peacock, H. L. Chapman, Jr., and R. E. L. Greene. 1974. Forage and supplement systems for beef cows in south-central Florida. Proc. Soil & Crop Sci. Soc. of Fla. 33:pp 56-59.

TABLE 2.--Hay supplement, weaning percentages, weaning weight, and weaned calf production on four forage systems at ARC, Ona, 1973-1976

	Supplemental			
Forage	hay per	Weaning	Weaning	Calf
System	cow, annual	rate	weight	production
	lbs.	%	lbs.	1bs/acre
1. Grass	315	80	481	237
2. Grass + extra N	185	88	505	278
3. Grass + ryegrass	423	80	505	252
4. Grass + ryegrass + clover	362	88	503	273

<sup>1/</sup> E. M. Hodges, F. M. Peacock, H. L. Chapman, Jr., and D. Crane. 1978. Forage systems for cow-calf herds in south-central Florida. Proc. Soil & Crop Sci. Soc. of Fla. in press.

A series of mini-forage systems were established at ARC, Ona, beginning in 1967 and weaning percentage, weaning weight, and calf data from this experiment appear in Table 1. Seven systems were established with breeding herds of 25 cows on 40-acre units and continued year-long. One unit, consisting of perennial grass supplemented with molasses, was stocked with 30 cows on 40 acres. The perennial grass was fertilized twice annually with 50-25-25 pounds per acre of N, P205, K20. The legumes received no nitrogen and ryegrass received additional N as conditions indicated. The molasses-supplemented perennial grass and the ryegrass-clover-sorghum systems produced the most calf poundage per-acre. Calf weaning weights were excellent on all treatments.

A second trial, covering four years, was established with four treatments, each of which included 50 breeding-age females. It was observed in the first system series that N was constantly in short supply and, accordingly, the base rate was adjusted to include two annual applications of 64 pounds/acre N in the second experiment. In addition, one treatment received 50% more N annually in a third application which was spaced between the early spring and the fall dates. The annual legumes were omitted from the second trial, not for lack of value but because of the limit on variables that could be handled.

The herds in the second experiment received only limited amounts of hay in some years. Data in Table 2 show weaning rates ranging from 80 to 88 percent with no statistically significant difference. These values represent a range substantially higher than in the preceding trial. Weaning weights were similar for all treatments and only slightly higher than those observed in the first trial. Production per-acre on the basic grass system was 11 percent above the earlier value, 237 pounds vs 213 pounds while the nitrogenfertilization rate was 28 percent higher in the second trial.

The similarity of results between systems indicates that orderly management can combine a number of different components into workable herd production systems. It has been a continuing observation that the intensive grazing systems require a reserve of stored forage to allow adjustment to wide fluctuations in weather and the consequent changes in forage supply.

#### LOOKING TO THE FUTURE IN FORAGE-ANIMAL PRODUCTION

by R. E. Blaser

Concerning this topic, it is precluded that the paper should deal with the animal-forage complex. However, the future progress depends on professionalism in research, teaching, and service. In the broadest sense, we professionals are charged with responsibilities for developing and implementing principles to manage the soil-biotic-climatic complex so farmers may encounter less risk and potentially more profit from ruminants. Consumers would also benefit.

I begin with praise for our many excellent accomplishments in a wide array of interplaying factors to advance ruminant production. A long list of varied citations would be very impressive, commendable, but incomplete. However, when compiling all costs for professional, technical, and semi-technical personnel, operations, etc. as compared to our accomplishments as individual professionals we would likely be either depressed or surprised. Have we professionals achieved high potentials of service and knowledge to advance forage and ruminant production?

We dislike evaluation, don't we? Each one of us should be required to evaluate our individual accomplishments yearly, writing out what new principles have been established that have advanced knowledge or been implemented into practice. Listing the accomplishments during the past five years and projected programs for the next five years could concurrently point out weaknesses and strengths and serve to stimulate new creativity and innovations for progress along with the joy of serving in our professions to benefit mankind through research, teaching, and service. A strong, continued, and dynamic professional growth to improve knowledge and services should be our mission.

At our university, promotions and salary increases depend on annual evaluations - assistant professors may draw higher salaries than professors. The drawing of large salary increases by proficient faculty and no increases by "nonperformers" is endorsed. After being asked how to develop a strong Institute of Research in Chile by Director Ellguetta, I suggested that salaries not be paid on the basis of age, sex, color, family name, degree, but on the basis of accomplishments substantiated by annual self-evaluations by each professional. After adopting this recommendation, Mr. Ellguetta was pleased to report that self-evaluations stimulated new innovations and improved the amount and quality of research by more than 30%.

We often "rehash" old work or pursue research where the results can be predicted. Many projects will have little impact on the livestock industry. Paper presentations often give little new information. The free and fixed so-called "hard" state and federal funds are assigned irrespective of accomplishments. Funding for salary, rank, and operations should be based on the effectiveness of our professional contributions, be they research, teaching, extension, or business pursuits. Would this stimulate creativity and performance? Some professionals have become "freeloaders" at state and federal levels.

Unless evaluations of professionals are sharpened, there will be a continued decline in proficiency and this is partly responsible for inflation and other problems in our society. In contrast, the so-called "soft" contract funds for research, teaching, and extension are in reality "hard" monies. Such funds awarded to innovative projects must be justified by meaningful interpretations of findings and recommendations for implementation.

In my opinion, protectionisms such as tenure and state and federal personnel acts, unions, and civil service concepts have promoted or tolerated mediocrity. Socialization is a serious deterrent to professional growth and service. To fulfill a pledge of reorganizing federal bureaucracies for efficiency, President Carter began by making all positions secure. Socialization continues with the federal law that allows persons to work until they are 70. We oldsters should be replaced by qualified young persons.

Lowering the standards for student enrollment and for professionals to accommodate minority groups is of grave concern. Historically and generally, the advancements in the United States in medicine, agriculture, industry, and other arenas have been directly associated with excellence in professionalism. Supporting roles are very important; thus, individuals with sub-professional qualifications, regardless of race, sex, or creed, can perform very useful services in such areas. Indeed, persons not qualified for the high standards of professionalism will undoubtedly be more contented and mentally stable in supporting roles.

It is noteworthy that there is little criticism about fairness in professionalism in male athletic programs. For example, the winning basketball teams at many institutions are allied with the excellent black professionals. No one is complaining when all persons on an athletic team are black, unless they lose. Competency should be of highest priority from the viewpoint of new innovations for continued advancements in society. Professional qualifications are not saddled with sex, race, or creed. Talents differ; we must recognize that supporting and leadership roles are always components of societies. Service, concern, and love should be interplaying ingredients among persons, all areas of employment being important.

Although it has not been true in the past, I believe the doors are now open for qualified professionals in any arena. Concerning women, we welcome them as co-professionals. About 1/3 of the students in my senior and graduate course in forage ecology and utilization are females, many being top "A" students and highly qualified persons.

At all levels, creativity, originality, and quality and quantity of output by professionals is often impaired by "overadministration" which depresses funds and productivity because of useless paperwork. Administrative confusion is of concern in many federal areas and entanglements in state and federally administrative programs often discourage professionalism. Strong extension specialists should be employed with freedom to do their "thing"; instead, the top and sub-sub-lines of administrators inhibit the quality of professionalism and progress by regimentation of programs. Will administrative arenas be reduced and simplified and a "professional trust" be reestablished?

#### RUMINANTS AND FORAGES

Ruminants of paramount economic importance since biblical times will continue to make formidible, economic contributions for food and clothing. As human populations increase, the best tillible soils will have first priority for

producing legume and grain cereals for direct human consumption. The nutritional aspects of cereals will be improved genetically or further fortified to satisfy the needs of human nutrition and health. However, rolling topographies make it necessary to use perennial grass-legume associations alone or in rotations to maintain or improve soil structure and organic matter, all serving to increase water infiltration to reduce erosion and improve fertility. Pending on costs of nitrogen and crop pest control, perennial grasses and legumes may again be used in rotations. Marginal stony and shallow soils will be used exclusively for forages. Ruminants will be used extensively for converting crop residues and animal wastes to food and other products. Financial or cyclic risks among cattle producers and a poorly informed public are serious unsolved problems.

## Ruminant Efficiency

Unfortunately, there is little specific information on the possibilities of developing races of ruminants that are efficient converters of forages to animal products. Letter queries seeking data on this topic have generally been tabled. The general opinion from replies state that ruminants with the highest feed conversion from grain-forage rations are also most efficient forage converters. Large "growthy" ruminants are generally superior to smaller compact types. Breeders and geneticists see little chance for improving the efficiency on forage diets since the lifetime diet of beef cattle is generally 70-80% forage. Unfortunately, registered and unregistered young bulls in testing programs are invariably fed high grain rations to measure genetic potentials and to promote sales.

With female cattle replacements, a reasonable goal is to select replacements restricted to forages, excluding corn silage. At Middleburg, females not fed grain since 1951 have averaged over a 90% weaned calf crop on herbaceous forages. Cows without a calf during any year go to the butcher. Longevity and productivity of our cows have been excellent, 12 calves during the life span being common. As compared to other herds, we do not know whether the Middleburg herd is more or less efficient on herbaceous forage diets. ently, buffalo and buffalo crosses have forage conversion rates similar to those of cattle. Apparently, there is no breed or type best for all conditions (8). The possibility of developing special cattle races highly efficient in converting forages is an important unanswered question and apparently of no immediate concern to animal breeders. Can intake of forage and digestibility in the rumen be augmented? Such high risk research should be investigated as an increase of 1 percentage unit in ingested digestible energy (IDE) of any forage would be of national and international importance for increasing production efficiency.

#### PROBLEMS AND NEEDS

We have prepared 5 review interpretations  $(\underline{1}, \underline{2}, \underline{3}, \underline{4}, \text{ and } \underline{5})$  with many references dealing with principles and philosophies for managing animal-forage systems. Thus, statements herein are not substantiated by references and I will discuss only a few of many factors dealing with ruminants in grazing regimes.

Insufficient ingested digestible energy (IDE) by ruminants from the summer growth of most perennial forages deters economic production of ruminants with high energy requirements. The efficiency of energy conversion from

forages for growing or finishing meat producing animals and lactating cows increases sharply as IDE is elevated. Supplementary grain feeding (energy) with herbaceous forages invariably increases rates of gain or milk production, but may or may not be economical. The need of supplementing energy to herbaceous forages depends on species, season, stage of growth, grazing pressure, and the ruminant category. Silages of grain varieties of corn and sorghum need not be supplemented with IDE. Conversion efficiencies of such silages are high, daily gains exceed 2 pounds and choice carcasses are commonly produced.

Ingested digestible protein, even for high producing lactating cows, is usually adequate with good management of temperate annual and perennial grasses and legumes.

The need for high yields, longevity, and high IDE of temperate grasses and legumes is critical. Because of new diseases and insects, the new varieties are superior but have generally maintained yield levels of varieties of several decades ago when some of the present pests were absent. The IDE values, under controlled management for old and new varieties of temperate species, are similar. There have been marked increases in IDE and yields of some semitropical forages.

Because of pest problems, the acreages of alfalfa in the South have declined and yields stagnated. A decade ago, some plant breeders under the auspices of industry indicated an early availability of high yielding hybrid alfalfas. Where are such hybrids? Working with temperate perennials embodies "tough" genetic problems requiring the best in new innovations. Natural selection processes have produced good varieties of tall fescue, orchardgrass, timothy, bromegrass, red clover, alfalfa, bluegrass, and perennial ryegrass. Hopefully, some of the turf type bluegrass and ryegrass varieties might be used in forage systems. As long as 10 years of research to isolate superior varieties of different species have often failed. To obtain superior varieties, it might have been more innovative and productive to seed potentially adapted species on many farms in different environments under judicious grazing. During 10 years, this would have resulted in rigorous natural selection for disease tolerance and other adaptive factors from multimillion populations and multienvironments. Alfalfa varieties were seeded on a given soil (6): a) where alfalfa had never been grown; and, b) immediately after alfalfa. Yields are substantially higher on the "new" land, many varieties being extinct where alfalfa followed alfalfa. This indicates that plant breeding should be conducted on "dirty" land to obtain disease and insect resistance. Germplasm from temperate forages should be sought with enthusiasm from various "old" field environments. This is not sophisticated, so it will likely not be pursued, even though nature has been more successful in developing turf and forage varieties than some plant breeders.

Perennial ryegrass in mixtures is the key to successful grassland farming in many countries with temperate environments. It was accepted that perennial ryegrasses were not adapted to humid, eastern USA. Bluegrass has been condemned, disced, burned, killed with herbicides, and replaced by taller grasses and clover. Yet, in the mid-Atlantic region, bluegrass-clover mixtures produce animal gains and products similar to those for tall grass-clover associations. Turf agronomists have found persistent bluegrass and ryegrass strains in "nature". Are there forage types of these two and other species in nature waiting to be made useful? Farmers need better bluegrasses, perennial ryegrasses, and reseeding annual ryegrasses to plug into forage systems for ruminants having high IDE requirements and for lengthening the grazing seasons.

Alfalfa with rhizomatous or proliferating roots are needed for 12-month

forage systems. Such morphological types would invade and regenerate stands after mismanagement or pest epidemics. Also, morphological branching roots would make alfalfa adapted to semi-poorly aerated soils.

In forage management and physiology, there are serious shortcomings. We need to investigate species and genotypes under flexible managements to lengthen grazing seasons, improve mid-season production in year-round grazing programes with minimum harvesting. New work indicates that alfalfa and red clover may be grazed during early spring and stockpiled with grasses for winter grazing, in functional systems. For spring seedlings, autumn simulated grazing of red clover has not depressed stands nor dry matter yields. Tall fescue, thought to be too aggressive for alfalfa, appears promising, but in new seedings, alfalfa subdues fescue. We have obtained 2 tons of stockpiled tall fescue during late August-November from nitrogen transfer from alfalfa.

Tall fescue is a fantastic plant, broadly adapted, suitable for flexible management and uses - turf, erosion control, and in forage systems. Highly rhizomatous genotypes with high fructosan contents and yield potentials that maintain chlorophyll and cell structure for photosynthesis and retention of soluble carbohydrates and proteins during low winter temperatures are needed. As cells rupture during winter, fructosans (nearly 100% digestible) leach, causing declines in digestibility from around 70% in November to 45% in March. Simultaneously, it is very important to develop tall fescue free of toxicity syndrome (s) that will also improve IDE during summer. Steers grazing N fertilized Ky 31 fescue during the spring-fall season averaged 0.91 lbs daily as compared with 1.70 lbs during the autumn-winter season.

Declines in daily gains during the summer season with controlled grazing pressures occur with temperate and semitropical species. Can legumes, management, other species, or varieties arrest such declines in animal production?

The best IDE and outputs per ruminant from herbaceous species in the Southern region occurs during the late autumn-early spring season from winter annual grasses and legumes and possibly tall fescue. Can the risk of poor growth and seasonal distribution be subdued?

In the southern region, the maintenance of legumes is a serious economic problem. There has been excellent legume renovation research; however, better economic methods of regenerating and maintaining legumes in temperate grasses and establishing winter growing grasses and legumes in semi-tropical grasses are needed for forage systems. Can we find red and ladino clover varieties and managements for natural reseeding and regenerating as with white clover? Is it possible to maintain temperate perennials or volunteer winter annuals and semitripical species in association for year-round grazing?

#### COOPERATION

Concerning the forage-ruminant complex, cooperation has often been deterred by departmentalization. Strong departments with highly qualified professional personnel are essential. However, departmentalization is not functional in nature. Natural or artificial soil-biotic-climatic complexes demand multidisciplinary analyses and action programs for economic ruminant enterprises. As professionals in different departments, we should cooperate and direct our efforts to advance ruminant production in various environments. Thirty years ago, in Virginia, a written cooperative dairy-agronomy grazing project with milk cows stated that the agronomy department would furnish the Ky 31 fescue seed. It is different today. However, there is often only superficial cooperation; personnel in few states and federal organizations have

model cooperative team research among professionals to serve the broad complex for economic production of ruminants. Cooperation for enjoyable progressive programs cannot be made functional with an "administrative hammer". However, administrators should employ professionals with vision and cooperative aptitudes.

Can we listen and hear each other in developing relevant research in the broad arena of ruminant production unselfishly? Not yet! In environments of professional diversity, where ruminants are departmentalized, do we invite cooperating scientists to discuss and debate vigorously and openly? I am convinced that advancements in ruminant production depend on wholesome cooperation among scientists in various disciplines. Farmers want ideas, the departmental source is not important. However, cooperation will not assure relevance in research or extension; dynamic creativity, idea sharing, and free debate among each professional is essential. Progress and quality depend on ideas. In cooperative endeavors with complex systems, full agreement among the diverse scientists should not be anticipated nor required.

### VISION, SYSTEMS, AND PHILOSOPHY

To speed up economic advancement of ruminant production, extension and industrial personnel should assemble known principles into forage-animal management systems of production for economic evaluation. Many known principles are not being implemented by extension and industrial personnel. Many farmers will cooperate and such results should be published. This may serve as an impetus for researchers to develop forage-ruminant systems. Developing systems and obtaining new information is not a research responsibility per se. a team of persons in research, extension, and industry might plan forage-animal management systems for economic evaluation on farms. For example, an extension consultant, Jorge Zubizarreta in Argentina, has implemented principles from our work into systems of many large farms. When calling at my office and referring to Research Bulletin 45 to discuss principles, he closed the booklet stating, "No, I know what's in there and we use it - what have you that's new that can be incorporated into forage-animal systems?" Farmers he advises are using the principles of creep feeding, creep grazing, and first and last grazing in management systems.

At a session of this group about 18 years ago, Brady Anthony referred to a publication (7) which stated that 4 month old calves restricted to the dam's milk gained .33 lbs as compared to 2 lbs when calves had milk and feed. This important factor to be plugged into systems has generally been ignored. Why have animal scientists generally not accepted or disproved this very important principle? But listen to a paragraph in a letter from Anthony,

"I have made rather extensive calculations on the milk production of beef cows relative to calf performance. Our data show that the nursed beef calf must receive an outside source of nutrients equal to its caloric intake for milk at 90 days of age if it is to continue to grow at a rate of approximately 1.7 - 2 lbs daily. After 90 days of age for the rapidly growing calf, the percentage of its daily feed supply from milk rapidly declines. This situation holds for all beef cows nursing calves. This means that after the calf is 90 days of age, its performance is primarily conditioned by the source of food other than milk."

We would be critical of published concepts and statements in the forage-animal complex. It is easy to be wrong in this complex enterprise. Defying that weaning weights of calves depend on the dam's milk supply and the bull has opened new horizons. Defying that silage must be at least 65% moisture and making 40 to 50% dry matter silage has led to high quality energy silages from corn and grain sorghum. Such energy silages with only urea-protein meal supplements are forage-animal systems for weaned calves, fattening and lactating cows. Beef cows do not deserve such energy forages. Many texts still refer to all silages as roughages.

### Fixed and Managed Experiments

Persons in departments with responsibilities and concerns to improve ruminant production should pursue objectives in the following areas:

- 1) Plant Phases, Ruminants Not Needed as Testers: Dedicated efforts to develop simple forage systems for various environemnts for year-round grazing and minimum harvesting that provide the nutritional needs for different classes of ruminants and cycles of production economically.
- 2) Ruminant Phases: To develop desirable ruminants for various environments (long-lived, high levels of health and reproduction, disease and pest resistance, efficient in forage conversion, desirable marketing qualities) through genetics and a broad spectrum of ecological sciences or factors.
- 3) Animal-Plant Phases: To develop and evaluate simple and economic ruminant-forage management systems for various environments for entire categories of production such as calf production through weaning, growing phase, fattening or milk production. The systems with beef cattle should embody year-round grazing with a minimum of harvesting, mechanization, and hand feeding.

These research areas provide opportunities for personal professional development through individual, intra-departmental or interdepartmental research. The missions are to obtain relevant findings to be plugged into animal-forage management (phase 3). Assembling factors and managing them in systems to establish principles and economic potentials on farms has generally been ignored. Farmers hunger for such operational packages.

The research philosophy for these phases embodies two categories: a) classical designs with fixed variables; and, b) managed variables. The classical experiments with fixed variables are relatively simple to conduct. They are said to be objective because judgement is excluded during the conduct of the experiment. The findings are usually narrow in scope, pertaining to fragments of the broad complex of ruminant production. Such experiments are replicated, analyzed statistically giving probabilities, and published in elite peer review journals. Many experiments in this category are useful, but discussions to advance knowledge and implementation are usually weak.

Designing experiments with managed variables requires dynamic judgement while experiments are in progress. Since judgement is exercised, managed experiments are said to be subjective, unreliable, biased. A farmer is a manager; he makes decisions daily on wise compromises for producing desirable products profitably. When pocketed dollars increase because of management, is this objective? The point is that most of us can be complacent; we are not stressed or possibly we are not as well versed as a good farmer. We tend to abhor management in experiments. Listen to three Georgians. McCormick, Hale and Southwell (9) were disappointed with fixed objectives when fattening steers on small grains. Listem to them, "The conditions followed in conducting the

3 phases of this study were necessarily fixed; whereas, commercial feeders may adjust operations and ultimately realize more profits." Wisely, they pointed out weaknesses in their data and elaborated on managements that farmers might have used. Note this was published in a bulletin, not in an elite journal. The writing of a strong bulletin showing how to implement findings into farm practices requires highly versatile and knowledgeable professionals. One relevant publication in this area may be equivalent to several publications in elite journals with fragmented data. If you believe this, tell your dean and peers, - I have.

McMeekan researched fat lamb and milk production in New Zealand. Literature reviews credit him with evaluating rotational with continuous grazing under constant stocking rates. McMeekan actually imposed managements to vary the nutrition needed for classes of ruminants for various cycles of production through pasture management. Stocking rates for rotational and continuous grazing were constant but varied within each to allocate the needed nutrition. He did not compare rotational versus continuous grazing per sehe imposed harvesting and management. Further, in "control grazing", he did not use a given number of days grazing and resting within a paddock. Cows were shifted from a rotationally grazed pasture when judged that IDE became inadequate for lactation. McMeekan also stressed ruminants during certain reproductive cycles rather than pastures. Thus, McMeekan's subjective (managed) experiments were highly objective, based on their worldwide influence on economic production of ruminants.

In year-round forage-ruminant systems to produce ruminant products profitably, management is important. At Middleburg, experiments with forage-cowcalf systems investigated raising beef calves through weaning. Given cows were restricted to each of 10 systems for 4 years. The production goals of weaning calves at 550 lbs, a 90% calf crop, and high calf production/A were realized. Two reasonably high stocking rates with year-round grazing for several systems were compared with grazing-hay feeding systems. For simplicity, there were only 3 fields in a system. Each system was managed independently to realize the highest and practical potential of the systems.

Grazing pressures ranging from low to high at a given moment mean ranges of high to low nutrition (energy and protein intake). The continuously high nutrition requirements of calves were maintained by opening gaps to a fresh pasture (creep grazing) whenever grazing pressures of pastures grazed by cows and calves become high. When the residual pastures grazed by cows become extremely short (very heavy grazing pressure and plant stress), the cows were shifted in with the calves on the creep grazed pasture. The cows and calves then grazed together until judgement (management) indicated that the grazing pressure again deterred calf growth. At this point, the creep grazing gap in the next fresh pasture was opened, cows again grazed the residue, etc. This alternate creep grazing and no creep grazing is managed to maintain low grazing pressures for calves to achieve high growth rates and weaning weight goals. Conversely, grazing pressures of cows vary sharply - a medium grazing pressure during a few weeks before calving until calves are 3 or 4 months old to provide milk and to stimulate estrus for early conception. Such grazing pressure-nutritional control for allocating quality forage to calves instead of cows is economic, allowing high stocking to increase calf gains/A without sacrificing gains/calf.

Creep grazing or creep feeding is of no value under low stocking-low grazing pressure regimes unless forage is of poor quality. The Alabama findings show better calf gains from bermudagrass-clover mixtures than from bermuda-

grass; the differences were not attributed to milk production. Calves, about 3 months old, require quality forage or grain along with milk from their dams for high IDE.

With managed forage systems, cattle can be finished to good and choice grades without grain; likewise, beef cows, ewes, stockers, and replacements do not need grain nor protein supplements with managed forage systems.

When evaluating varieties, it is often recommended to use 3 stocking rates and several replications. Management in such experiments can save space, time, and money. For example, when ascertaining milk production potentials from Ky 31 versus Kenwell fescue or liveweight gains from Ky 31 versus Kenhy tall fescue, one controlled (managed) grazing pressure was adequate for evaluation and to show severe fescue foot of cattle grazing Kenwell and Kenhy varieties at Middleburg.

Usually, in research and service, farmers get fragments of information. We need to help them by planning forage-ruminant management systems for entire economic phases of ruminant production. Managing the interplaying factors and controlling and allocating IDE to appropriate ruminants has tremendous economic potentials. Managements should be planned to recycle animal excreta to increase forage yields. Also, cows during certain stages of reproduction may be used to replace "machines" as for grazing rather than mowing weeds, and very high grazing pressures to reduce grass competition when reestablishing legumes.

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# BREEDING AND SELECTING LEGUMES FOR GREATER N2-FIXATION AS SEEN BY A MICROBIOLOGIST

By Harold L. Peterson

#### INTRODUCTION

The probability of successfully enhancing biological dinitrogen fixation by Rhizobium spp in association with plants from the family Leguminosae has perhaps never been greater than it is today. Our understanding of symbiotic dinitrogen fixation has expanded tremendously during the last decade. Major advances in the biochemistry, genetics and physiology of N $_2$ -fixing symbioses have set the stage for significantly increasing dinitrogen fixation.

Recent attention has been focused on the potential of genetic engineering in  $N_2$ -fixation (Hollaender, 1977). While this concept is not new, it has successfully stimulated the imaginations of many persons in the private, business and government sectors of society. Indeed, long range improvements in the  $N_2$ -fixation process may depend on successful transfer of procaryotic "nif" genes to eucaryotic organisms. However, immediate and intermediate range improvements in  $N_2$ -fixation will certainly depend on accelerated selection and breeding of rhizobia-legume combinations for increased dinitrogen fixation. This problem has been addressed since the discovery that bacteria in legume nodules fixed atmospheric  $N_2$  for the plant, but recent progress in several areas of research may remove some of the obstacles that have hindered development of these rhizobia-legume combinations.

This paper will review recent procedures that show promise in helping plant breeders and rhizobiologists select forage legume-rhizobia combinations for enhanced dinitrogen fixation. The discussion will concentrate on the rhizobial aspect of the symbiosis. Special attention will be given the challenge of establishing superior  $N_2$ -fixing combinations of Rhizobium spp and forage legumes in the field, and how selection and breeding can contribute to successful establishment.

# RHIZOBIUM - THE N2-FIXING MICROSYMBIONT

#### Characteristics

Bacteria belonging to the genus Rhizobium Frank (kingdom Procaryotae, Division Bacteria, Order Eubacteriales, Family Rhizobiaceae) are differentiated because of their ability to nodulate leguminous plants, and presumably fix  $N_2$  within these nodules. These small, motile, pleomorphic rods are non-spore forming and gram-negative. When grown on media containing substantial amounts of carbohydrate, Rhizobium often produce a great deal of extracellular polysaccharide (slime) and may develop cytoplasmic inclusions of poly-B-hydroxybuty-rate. They are aerobic, but can tolerate  $0_2$  tensions less than 0.01 atm. Optimum temperature for growth varies from 25 to 30 C, and pH from 5.0-8.5

(Buchanan and Gibbons, 1974).

Rhizobia are often separated into two groups according to growth rate on yeast-extract containing media, and the type and number of flagella. The fast growers form distinguishable colonies on yeast extract - mannitol (YEM) agar in 3 to 5 days; numbers of flagella vary from 2 to 6, and occur at lateral positions on the bacterium (peritrichous) arrangement. Species within this group are R. leguminosarum Frank, R. phaseoli Dangeard, R. meliloti Dangeard and R. trifolii Dangeard. Fred et al. (1932) indicated that Rhizobium leguminosarum usually form nodules with species of Pisum, Lathyrus, Vicia, Lens and Cicer. Rhizobium phaseoli nodulate species of Phaseolus, and R. meliloti species of Medicago, Melilotus and Trigonella. Rhizobium trifolii form nodules on Trifolium spp.

The slow growers typically form colonies < 1mm in diam on YEM agar in 5 to 10 days; a single flagellum (monotrichous) may be present at polar or subpolar sites on the bacterium. Two species are recognized: Rhizobium japonicum (Kirchner) Buchanan and Rhizobium lupini (Schroeter) Eckhardt.

Other strains of Rhizobium, mostly slow growing, are combined in a composite group called cowpea rhizobia. Glycine spp are usually nodulated by R. japonicum, whereas R. lupini nodulates species of Lupinus and Ornithopus. Cowpea rhizobia nodulate many genera of legumes such as Acacia, Arachis, Baptistia, Cassia, Cajanus, Crotalaria, Desmodium, Dolichos, Genista, Lespedeza, Phaseolus, Pueraria, Stizolobium and Vigna (Fred et al., 1932).

Vincent (1977) has recently reviewed the characteristics and complexities of <a href="Rhizobium">Rhizobium</a>, and the reader is encouraged to consult his paper for an excellent and comprehensive discussion.

#### Isolation

The procedures used to obtain strains of rhizobia usually involve isolation from legume nodules. Although selective media have been reported for direct isolation of rhizobia (Greig-Smith, 1912; Graham, 1969), attempts at direct isolation from soil have failed.

Vincent (1970) summarized the general procedure for isolation of rhizobia from soil. Briefly, a legume is grown in contact with a selected soil and nodules are allowed to develop. Representative nodules are removed from the roots, surface-sterilized, and tissue containing rhizobia is transferred aseptically to petri plates containing a sterile agar. 'Rhizobia-like' colonies are cloned by repeated transfer until representative isolates from single-cells are presumably obtained. These colonies are transferred to slants and maintained in a fresh and highly viable condition. Finally, isolates are verified as rhizobia by inoculating the host legume and growing the legume under rhizobially controlled conditions, examining for the formation of root nodules. New isolates of Rhizobium are characterized bacteriologically and catalogued for further use.

The time involved from initial isolation through characterization can involve as few as 10 weeks with  $\underline{R}$ .  $\underline{\text{trifolii}}$  to 6 months with slow growing cowpea rhizobia, assuming of course that everything goes according to plan. Unfortunately, problems with rhizobial contamination, growth chamber failure, leaky greenhouses etc. can double or even triple the amount of time required to obtain a new strain of  $\underline{Rhizobium}$ .

Unfortunately, current isolation procedures are very inefficient in

obtaining strains of Rhizobium differing markedly in N2-fixation. For example, we obtained 195 isolates of 'rhizobia-like' bacteria from nodules for Trifolium pratense L. CV. 'Kenland', Trifolium incarnatum L. CV. 'Tibbee', and Trifolium vesiculosum Savi., CV. 'Meechee'. All isolates were cloned and reinoculated on the host legumes, yielding 129 strains of R. trifolii. Analyses of plant dry matter and acetylene reduction (still in progress) suggest that N2-fixation by only three strains differed significantly from the overall mean. All three of these strains are ineffective.

Strains of Rhizobium vary considerably in a wide range of characteristics, including morphology, physiology, serology and ecology. Many studies have reported variations in these characteristics (Vincent, 1977). A detailed reiteration is not within the scope of this paper, and the reader is referred to the review by Vincent (1977) for further details.

#### GENETIC VARIABILITY

The nodulation process is controlled genetically by both the leguminous plant and the rhizobia, with the plant exerting perhaps the greater controlling influence (Nutman, 1969). Despite the abundant phenotypic evidence implying extensive genetic variability within natural populations of rhizobia, little progress has been made in genetic mapping of Rhizobium spp, especially in relationship to nodulation and  $N_2$ -fixation.

Genetic variability among rhizobia would seem to be partially responsible for the host-plant specificities that have been noted in many of the forage legumes. In the light of Vincent's (1977) discussion, it seems probable that this specificity occurs in many, if not all, of the steps involved in the nodulation process.

Certainly, the stimulation of <u>Rhizobium</u> spp in the rhizosphere of leguminous plants has been documented (Nutman, 1965, 1969; Vincent 1974; Dart, 1974, 1977; Parker et al., 1977). However, the biochemical (not to mention genetic) explanation is lacking for the preferential stimulation of certain strains in the rhizosphere. Rhizobia compete with other soil microorganisms (including other rhizobia) for available organic and inorganic nutrients in soil. Legume root exudates enhance the growth of <u>Rhizobium</u> spp in the rhizosphere (Dart, 1974). Parker et al. (1977) have shown that coldwater extracts of soil can support the multiplication of <u>R. trifolii</u> and <u>R. lupini</u> from an initial density of  $10^5$  viable cells/ml to a final density of  $2-4 \times 10^9/\text{ml}$ . These extracts were obtained from a l:l water-to-soil ratio (v/w). Competition is important in suppressing rhizobial growth and may be very intense in soils of the southern U.S. where numbers of rhizobia rarely exceed  $10^4/\text{g}$  (Peterson, unpublished).

The infection process is another area where genetic variability among strains of Rhizobium influences the processes of nodulation and N2-fixation. Nutnam (1953) found that strain C13R of R. trifolii lost the ability to nodulate  $\underline{\mathbf{T}}$ . Purchase (1953), Purchase and Nutman (1957) used a non-nodulating strain of R. trifolii to inhibit nodulation of red clover by a normal virulent strain.

Trifolium ambiguum is another interesting example involving a host-strain interaction limiting nodulation. Parker et al. (1949), Parker and Allen (1952) found that  $\underline{T}$ .  $\underline{ambiguum}$  rarely forms nodules with most strains of  $\underline{R}$ .  $\underline{trifolii}$ . Hely (1957) found that strains of  $\underline{R}$ .  $\underline{trifolii}$  from Turkey were much more efficient in nodulating  $\underline{T}$ .  $\underline{ambiguum}$  than strains of  $\underline{R}$ .  $\underline{trifolii}$  isolated from soil in New Zealand.

More recent work may be providing an explanation for the inabiltiy of certain strains of Rhizobium to infect leguminous plants. Dazzo and Hubbell (1975) established that cross-reactive antigens were present in cell walls of compatible combinations of R. trifolii and T. repens CV. 'Louisiana Nolin' and T. fragiferum L. CV. 'Salina'. If a strain of R. trifolii lost infectiveness, a portion of the antigenic homology was also lost. Antigenic homology was absent between T. repens and isolates from the other Rhizobium spp.

A clover lectin was isolated that would bind infective but not noninfective strains of  $\underline{R}$ . trifolii. The lectin facilitating the agglutination is sensitive to acid, alkali, pronase, trypsin, periodate and urea, suggesting that the material is protein (or glycoprotein).

Dazzo, Napoli and Hubbell (1976) found that noninfective strains of  $\underline{R}$ .  $\underline{\text{trifolii}}$  and strains of  $\underline{R}$ .  $\underline{\text{melioti}}$  are absorbed in similar yet small numbers by  $\underline{T}$ .  $\underline{\text{repens}}$  root hairs. However, infective  $\underline{R}$ .  $\underline{\text{trifolii}}$  cells were absorbed in numbers nearly five times greater. A 2-deoxyglucose-sensitive receptor site was implicated as the molecular point of coordination present in both clover roots and rhizobial cells. Further verification of these findings has been provided by Dazzo and Brill (1977).

Genetic variability within the legume host and rhizobia is probably also expressed in the formation of infection threads. Work by Lim (1963) indicates that infection is proportionally related to the number of rhizobia present in the rhizosphere. However, when the first nodule is formed, an increased number of bacteria are required to promote futher infection. Purchase and Nutman (1957) noted that formation of the first nodule in  $\underline{\mathsf{T}}$ . pratense resulted in much larger numbers of rhizobia being required for the formation of additional nodules. The reasons for inhibition of further nodulation are unknown.

Hubbell et al. (1978) found that strains of Rhizobium spp that typically infect the legume root via root hairs can produce low levels of pectinolytic enzyme activity. This finding is very important since it reinforces Nutman's (1956) "invagination" hypothesis of infection. It also represents another source of variablity, and may explain why some infection threads abort. If the outer cell-wall of a root hair must be removed enzymatically in close coordination with the growth of the infection thread, variation in pectinase activities of the rhizobia may be very important in determining whether or not the infection thread will reach the root cortex. This may explain the observation of Nutman (1949) that infection threads do not form if rhizobia penetrate the lumen of the root hair.

Once the nodule has formed, genetic factors in both rhizobia and plant govern the effectiveness of the N<sub>2</sub>-fixation process. Holl and LaRue (1976) have listed the plant genes known to regulate N<sub>2</sub>-fixation in several legumes, including  $\underline{\mathbf{T}}$ . Pratense. Within natural populations of Rhizobium, individual organisms vary considerably in N<sub>2</sub>-fixation capacity. In  $\underline{\mathbf{R}}$ . trifolii, Bergersen et al. (1971) found significant variation among isolates in effectiveness of N<sub>2</sub>-fixation with sampling areas and sampling years. The effectiveness of most  $\underline{\mathbf{R}}$ . trifolii isolates from three of four sites in southeastern Austrailia on  $\underline{\mathbf{T}}$ . subterraneum was only 70 to 85% as great as the control (strain TA1). Approximately 57 of 420 isolates exceeded TA1 in effectiveness of N<sub>2</sub>-fixation as determined by dry-matter accumulation. The remaining isolates were only 20-90% as effective. Natural isolates of  $\underline{\mathbf{R}}$ . trifolii from 7 locations were only 78% as effective in fixing N<sub>2</sub> as strain TA1. Gibson et al. (1975) found substantial variability in effectiveness of  $\underline{\mathbf{R}}$ . trifolii isolates from eight regions of southeastern Australia over a five year period. As in the work reported by

Bergersen et al. (1971) most isolates were inferior to TA1 in fixing N<sub>2</sub>.

Despite abundant phenotypic evidence implying extensive genetic variability to nodulation of N<sub>2</sub>-fixation within rhizobia, little progress has been made in genetic analysis and mapping of Rhizobium spp. The main handicap to understanding the genetics of N<sub>2</sub>-fixation is a lack of techniques for analyzing phenotypic expression by rhizobia without using leguminous plants (Swinghamer, 1977). This restriction may soon be removed, because Tepkema and Evans (1975), Pagan et al. (1975), Kurz and LaRue (1975) and McComb et al. (1975) have obtained N<sub>2</sub>-fixation (or C<sub>2</sub>H<sub>2</sub> to C<sub>2</sub>H<sub>4</sub> reduction) in pure cultures of several Rhizobium spp. The stage has been set for some very exciting and important breakthroughs in biological N<sub>2</sub>-fixation by the Rhizobium-legume symbiosis.

# SCREENING STRAINS OF RHIZOBIUM FOR N2-FIXATION WITH LEGUMINOUS PLANTS

Classical tests for compatibility between rhizobia and leguminous plants are based on the extent of plant growth when inoculated with a strain of rhizobia and grown in a nitrogen-free media. Usually these tests are conducted in the greenhouse or growth chamber under 'rhizobially-controlled' conditions. An effective strain of rhizobia is usually included as a positive control so that the relative capacity for  $N_2$ -fixation can be established by the unknown strain(s). This type of test is more valuable for forage than grain legumes because N-content and dry matter production are more closely related in forage legumes (Erdman and Means, 1952), and the onset of seed production in forage legume does not seem to dominate plant activities as extensively as in grain legumes (Burton, 1976).

Screening can be carried out in a variety of containers such as tubes, crocks, bottomless-bottles (Burton, Martinez and Curley, 1972; Gibson, 1963) and plastic pouches (Weaver and Frederick, 1972). Unfortunately, determining the N2-fixation potential of a strain requires that growth continue until significant differences appear in nitrogen content or dry matter production among plants. The time required for these differences to appear varies considerably among legume species. Gibson et al. (1975) were able to separate  $\underline{R}$ .  $\underline{trifolii}$  isolates using  $\underline{T}$ .  $\underline{subterraneum}$  CV. 'Bacchus Marsh' at 31 days after planting. Large-seeded legumes may require more time to overcome the initial influence of nitrogen in the seed.

An important new screening technique was introduced by Wacek and Brill (1976). They developed a rapid assay for screening  $N_2$ -fixation ability of soybean cultivars and rhizobia. Inoculated seeds are planted in 20 ml serum bottles containing sterile vermiculite and plant growth solution. A sterile plastic bag is placed over each bottle and loosely fastened to permit gas exchange. After 14 days growth, the shoot of the plant is removed and the container stoppered.  $N_2$ -fixation is then measured via an acetylene-to-ethylene reduction procedure. The technique has recently been applied to screening forage legumes and strains of <u>Rhizobium</u> for effectiveness of  $N_2$ -fixation (Maier and Brill, 1976).

Plant tissue culture is another system that may be applicable to rapid screening of rhizobia for  $N_2$ -fixation. Child and LaRue (1976), for example, developed a tissue culture system that was used to determine nitrogenase activity of rhizobia within 14 days of inoculation. Interestingly, Child and LaRue see little advantage in using tissue culture for routine screening analyses. They indicate that more time, expense and energy were involved with tissue cul-

ture than in standard grow-out procedures, an assertion that may be somewhat debatable.

Pierce (1978) has described work of Dr. Don Barnes and colleagues on improving N2-fixation by  $\underline{\text{Medicago}}$  sativa L. and  $\underline{\text{Rhizobium}}$   $\underline{\text{meliloti}}$ . Dr. Gary Heichel is developing a new growth chamber system for simultaneous, non-destructive, whole-plant studies on photosynthesis and N2-fixation. It is hoped that characterization of CO2 exchange, acetylene-to-ethylene reduction and N15 uptake will help define the relationship between the photosynthetic capacity of legumes and the N2-fixation capacity of rhizobia. This should lead to improved legume-rhizobia combinations that are capable of enhanced N2-fixation and protein production.

We have developed a plastic-pouch procedure that can be used to measure  $N_2$ -fixation ( $C_2H_2$  to  $C_2H_4$  reduction) activity of nodulated forage legumes (clovers) (Peterson, in preparation). The technique involves growing inoculated plants in plastic pouches similar to those described by Weaver and Frederick (1972). A vacutainer needle is placed in the open end of each pouch. The pouch is then sealed to the stems of the plants (and vacutainer needle) using a material developed for sealing automobile windshields. Air is removed from the pouch and a known volume of a 90:10 mixture of (Ar +  $O_2$ ) and ( $C_2H_2$ ) is injected into each pouch. Ethylene production is determined using a gas chromatograph with  $C_2H_2$  serving as an internal standard. The principal advantages of the technique are that it requires very little growth chamber space, contamination by exogenous rhizobia is reduced, and plants may be analyzed repeatedly without apparent damage to either the plant or rhizobia in the nodules.

# THE ULTIMATE TEST - ESTABLISHING SUPERIOR RHIZOBIUM-LEGUMINOUS PLANT COMBINATIONS IN THE FIELD

Laboratory and greenhouse screening can be used to select rhizobialeguminous plant combinations with greater potential for  $N_2$ -fixation. But until we produce forage and grain legumes on a commercial basis in greenhouses, the ultimate test is the ability of a selected combination to perform in the field (Burton, 1976; Date, 1976; and many, many others).

Soil, unfortunately, is a rather inhospitable environment for both leguminous plants and rhizobia. Factors that effect the survival of both plant and rhizobia include soil temperature, moisture, pH, drainage, nutrient availability, pesticides and other additives, pathogenic and antagonistic microorganisms, presence of beneficial microorganisms, management, and time. Each of these factors has received considerable attention in recent reviews (Gibson, 1976, 1977; Mulder et al., 1977; Parker et al., 1977, Munns, 1977; Sprent, 1976).

Perhaps our concept of centers of excellence in research may need a minor modification. Plant-rhizobia combinations that prove successful in one region of the United States may fail in another region. This shortcoming can be avoided if the scientists performing the greenhouse and growth chamber screenings will employ conditions that represent the extremes encountered by the plant in its cultivation throughout the country. Also, as combinations are developed, field testing should be conducted at representative sites where the legume is grown and not confined to the region where the combination was developed.

In our work with annual clovers, three problems are receiving priority. The first problem is isolating and collecting strains of R. trifolii that are compatible with lines of T. incarnatum, T. vesiculosum and T. subterraneum being developed by Dr. W. E. Knight. This involves requisitioning cultures from throughout the world using the literature as a guide for strain selection, and the soon-to-be-revised IBP World Catalogue of Rhizobium Collections (Allen and Hamatova, 1973) as a source index. Strain isolation has proven a time consuming and frustratingly inefficient process. Work is underway to develop improved procedures for isolating superior N2-fixing strains of R. trifolii from natural populations. Hopefully, a new procedure will be perfected soon for use in the isolation process.

The second problem is coupling the legume-rhizobia selection process (winter annuals) with the reality of a field management system that usually requires incorporation of a cool-season grass with the legume for adequate late-fall and winter grazing. Strains of Rhizobium must be selected that will nodulate clover in the presence of nitrogen fertilizer (ie. 20-60 kg N/ha) used to promote early growth of the grass.

The third problem is developing inoculation materials and procedures that will insure maximum nodulation in the field by the superior strains of Rhizobium. On the surface this problem seems so basic that most people assume it has been solved. However, Weber (1977) has recently pointed out that the technology has not been perfected that will insure nodulation by an inoculated strain in soils already containing a population of host-infective rhizobia. The problem is aggravated in Mississippi because winter-annual clovers are usually planted during late August or early September. Soils are usually dry, and seeds may be subjected to temperatures of >40 C during a 1 to 2-week period after planting. Seed-borne rhizobia die very rapidly when exposed to temperatures greater than 40 C (Kremer and Peterson, unpublished).

Much work remains to be done. Hopefully through close cooperation between plant breeders and rhizobiologists, superior  $N_2$ -fixing Rhizobium-legume combinations can be obtained to help provide the high quality forages necessary for production of animal protein.

#### SUMMARY

Close cooperation between legume breeders and rhizobiologists should result in the development of plant-rhizobia combinations that can fix greater amounts of  $N_2$  from the air. In achieving this goal, the breeder and rhizobiologist must continually interact in the selection process. New, more rapid and sensitive screening procedures being developed hold great promise in allowing efficient and rapid selection of superior  $N_2$ -fixing combinations. Field testing is the final and perhaps most important step before a plant-rhizobia package can be recommended for adoption by producers. The main problem with field inoculation of legumes (following nearly 80 years of work) is lack of an inexpensive, dependable inocula to insure nodulation by the inoculated strain (s), especially in thermic soils that already contain less-effective, infective rhizobia. The current emphasis on genetic engineering for improved  $N_2$ -fixation will be an exercise in academic futility unless efforts are made to concurrently develop improved inoculation procedures and improved understanding of the ecology of rhizobia in soil.

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PANEL DISCUSSION: BREEDING GRASSES AND LEGUMES FOR USE IN MIXTURES

INTRODUCTION: COMPLEXITY AND CHALLENGES

By Pryce B. Gibson

Our panel is concerned with the forages that are grown in mixed stands. Usually these mixed stands are harvested by grazing livestock. Therefore, we are concerned with a plant community instead of one species and we are concerned with an important biological entity—the grazing animal. Obviously, the plant community and the grazing animal contribute variables to research involving the crops. I believe it is self evident that the complexity of research usually increases with the addition of variables and, therefore, research on the forage crops used in mixed stands is more complex than research on the crops used in pure stands.

Most mixed stands of forage crops involve a grass and a legume. species are intimately associated, mutually affect the microclimate, and compete for the essentials for growth. Consequently, the ecological interactions of a mixture are more complex than those of a monoculture. Contributions of the partners to the environment and the performance of the mixture are partly competitive and partly complementary. Legumes supply biologically fixed nitrogen, increase the quality of the forage, and may improve the seasonal distribution of production. Grasses supply fiber that is needed in the ruminant's diet, reduce the chance of bloat, reduce trampling damage, provide the legume some protection from low temperatures, and may, by serving as a trap crop, reduce the number of some nematodes. The complicated ecological interactions of mixed stands indicate that the true test of a cultivar is its performance in a mixed stand that is subjected to the stresses and interactions caused by normal use. Obviously, the relative importance of testing a cultivar in a mixed stand rather than in monoculture may vary with annuals, perennials, cool season crops, warm season crops, location and management.

The success of a grass-legume mixture depends upon the mutual compatibilities and contributions of the two species. The two are a team and each species should have characteristics compatible with the needs of the other. A draft horse and a race horse make a bad team regardless of the fine breeding of each because the members of the team are not compatible. Maximum aggressiveness that may be a desirable attribute of a forage for growth in a monoculture may be objectionable for growth in a mixture. The success of a mixture depends on the breeder of each species considering the characteristics needed for success of the mixture, not for supremacy of his species.

I have encouraged panel members to comment on the following statements and questions: (1) "Forage breeders should make more selections and conduct more strain tests in mixtures as the crops are used, thereby testing the

compatibility of the entries and the ability of the legumes to supply nitrogen for both crops". (2) "Should breeders of pasture species use grazed areas in lieu of cultivated space planted nurseries and by so doing evaluate plants under actual pasture conditions?" This approach would substitute the grazing animals for some labor and is particularly applicable to breeding for improved persistence of stands under grazing. Unfortunately, implementing this approach is difficult and the plant breeder must control time of grazing. However, once implemented this approach may be more efficient than traditional methods. Also, I have suggested that each plant breeder include remarks on:

1. Extent of crop use in mixture vs in pure stands.

2. Advantages and problems of mixtures vs pure stands from the standpoint of forage production and utilization.

3. Comments relative to breeding and testing (e.g.: What are the plant characteristics and other components that affect the success of the mixture and the species in the mixture? What consideration of compatibility for growth with other species should be included in the breeding program?).

Partly because forage research involves several species and several variables, procedures and priorities used in forage breeding vary. Consequently, differences in opinions exist and probably will surface in our presentation. Although the make-up of our panel is diverse, our topic should be of interest to all concerned with forages. If alloted time permitted we logically should have included an entomologist and a plant pathologist to discuss insects and diseases in mixtures vs in pure stands. Because our time is limited, we are depending upon our crop ecologist and other panel members to consider insects and diseases.

PANEL DISCUSSION: BREEDING GRASSES AND LEGUMES FOR USE IN MIXTURES

SUMMARY OF ECOLOGICAL CONSIDERATIONS
IN RELATION TO THE BREEDING AND DEVELOPMENT OF
LEGUME CULTIVARS WHICH CAN BE GROWN IN GRASS-LEGUME MIXTURES

# By O. Charles Ruelke

Ecological considerations are the first and probably the most important considerations in breeding legumes which can be grown successfully in grass-legume mixtures. Until specific species of legumes and grasses are found which are compatable in mixtures, efforts to improve either the legume or grass have little chance for success. However, breeding for compatability and persistence of legume and grass species which have a potential in mixtures, can greatly enhance the performance of each species and result in far better production from the grass-legume mixture than from either species grown alone.

Many factors of the environment need to be considered when establishing grass-legume mixtures. First, and most important, is the temperature factor. No single species of grass or legume can be expected to be productive in the middle of the hot summer and also the middle of the cold winter. However, with proper selection of certain perennials and or self-seeding annuals, maximum production is possible, either during the warm or during the cool season. It is also possible to select species to produce alternately, namely, bahiagrass in the warm season and white clover in the cool season. Many combinations can and need to be considered.

Proper soil moisture like temperature can be the critical and or deciding factor in selecting and breeding grasses or legumes for mixtures.

Soil fertility often limits certain species from mixtures and the competition between species for the nutrients often determines if or which species will persist.

Light intensity and photoperiodic response are also very important from the standpoint of establishment of the reseeding species as well as to whether an established species will flower and make seed.

The biotic factor including the harvesting by man, animals, caterpillars, or parasitism by plant diseases are factors of significant importance

Ultimately, it is the effects of one plant on another that limits the production of a mixture. It is this effect that the breeder must deal with.

In pure stands of a single species, most of the plants are bred to germinate and emerge at the same time. In mixtures one species may germinate or resume growth earlier than another, thus taking up the moisture and nutrients or forming a canopy to reduce the light on the other species.

Plant growth habit, which may be erect to prostrate, can be altered by breeding so that species compete more or less. Leaf angle, which may be acute or obtuse, can be altered which may increase the efficiency of use of light. Presence or absence of pubescence or waxy leaf coat can be altered by breeding which could alter water loss rates, or insect and disease resistance. These are but a few examples of ways the plant breeder could alter legumes for use in mixtures.

Finally, the growth habit, growth rate, and recovery rate of any one of the species in a mixture can be altered by management. Therefore, the testing of any new breeder lines in mixtures should be done by clipping trials, to simulate use as green chop or hay, as well as by grazing animals to evaluate the response under grazing.

# IMPORTANCE OF MIXED STAND EVALUATION IN BREEDING AND VARIETY DEVELOPMENT--ANNUAL LEGUMES 1

# By W. E. Knight

In 1960, Henson and Hollowell listed over 40 species of winter annual legumes adapted to the South. A number of these species that were of economic importance between 1945-1955 are no long available commercially.

Most annual legumes grow well in pure stands or in mixtures. The winter annual clovers are grown primarily in mixtures with ryegrass and/or cereals on prepared seedbeds and overseeded on permanent grass sods. Vetches may be grown in mixtures but have been used primarily as cover crops. The lupines are used for grazing and soil improvement and are usually grown in pure stands. When grown for grazing, they may be seeded alone or in mixtures with cereals.

At one time, an estimated 6-7 million acres of annual lespedezas were grown in the region. Most of this acreage was in mixed stands with summer perennial grasses.

Results of a survey completed in 1977 indicate a renewed interest in legumes and an increased breeding effort (Table 1). This survey indicates breeding or selection in 16 annual legume species.

According to Johnson and Byer evaluation of forage species to be grown in mixtures is complex and the researcher is faced with the problem of breeding for compatibility. A multidisciplinary approach to breeding and evaluation is necessary for rapid progress and essential to produce the forage legumes needed in a grass-legume system. The present demand for, and potential use of, improved forage legumes demands the initiation of coordinated improvement programs among plant breeders, pathologists, entomologists, microbiologists, soil scientists and seedsmen to meet the There is a lack forage legume needs of the livestock industry. of definitive information on fertilizer requirements of grasslegume pastures as related to persistence, quality, and interactions of applied nitrogen with legume Rhizobia including the interactions of sulphur and molybdenum as related to rhizobium efficiency.

The evaluation phase of the breeding program should seek answers to the following:

1. What management practices are necessary for establishing and maintaining grass-legume mixtures?

Cooperative investigations of the Mississippi Agricultural and Forestry Experiment Station and the Plant Science Laboratory, SEA, FR, U.S. Department of Agriculture, Mississippi State, Mississippi 39762.

Forage legume species in public improvement programs in the Southeastern United States Table 1.

													ļ
Legume Species	AL	AR	臣	G.A	KY	LA	MS	NC	Š	SC	NI	XI	VA
Alfalfa	B,R	B,R	B,R					B,R	В				В
Clovers:													
Arrowleaf	В		В	В			В						
Ball	B,R												
Berseem							S,R						
Crimson					B,R		B,R						
Kura					S,R								
Mike					S,R								
Persian													
Red			В		B,R	В							В
Rose					S.R								
Subterranean			В	В			B.R						
White	В		B,R			В	`			B.R			В
Zig Zag					S,R								
Lespedeza:													
Annua1		В											
Sericea	B,R				S,R								
Lupine		B,R		B,R									
Other Legumes:													
Alyce Clover			B										
Aeschynomene			Ω										
Desmodium			S,R	В									
Dolichos lablab													
Perennial Peanut			S,R,										
Siratro			ω										
Pigeon Peas			ß										
Stylosanthes			В										
Trefoil	B,R			S,R,	S,R,								В
Vetch	В				S,R								
2		-	1000										

1/ Results of a survey completed in 1977.

B = Breeding program in progress.S = Evaluation and selectionR = Release probably within 5 years.

- 2. Can minimum tillage practices be used successfully to establish new stands and reestablish clovers when stands are lost? (Non-point pollution).
- 3. What soil fertility management is necessary for optimal nutritional forage quality including fertility interactions as related to legume survival and rhizobial efficiency?
- 4. What are the causes of stand failures and lack of persistence (insects, diseases, nematodes, etc.)?
- 5. What is the potential of supplemental water as related to maintaining production, quality, and persistence and in regard to timely establishment of fall seeded crops?
- 6. What is the potential for reseeding as compared to maximum utilization and annual reseeding?
- 7. What is the economic advantage, if any, of a harvested seed crop from a grass-legume system?

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PANEL DISCUSSION: BREEDING GRASSES AND LEGUMES FOR USE IN MIXTURES

# BREEDING ANNUAL GRASSES FOR USE IN GRASS-LEGUME MIXTURES

By C. E. Watson, Jr.

Cool-season annual grasses, including ryegrass, wheat, oats, rye, and triticale, are widely used for winter pasture in the southeastern United States. Annuals provide an excellent source of high quality forage during periods when warm-season perennials are dormant. These species are grown alone or in combination with legumes on prepared seedbeds or permanent grass sods. Annuals are very aggressive species and are highly competitive with legumes in mixed swards.

#### ADVANTAGES OF MIXED SWARDS

Several benefits are realized by growing annual grasses with legumes. These include:

- 1. The legume component can fix nitrogen symbiotically.
- 2. The inclusion of a legume provides improved forage quality over pure grass.
- 3. There is less chance of bloat.
- 4. The fibrous grass root system makes for better footing, less trampling damage, and may prevent some heaving damage to the legume.
- 5. The legume reduces the tetany potential.
- 6. There is a better seasonal distribution of yield.

#### DISADVANTAGES OF MIXED SWARDS

Among the disadvantages associated with grass-legume mixtures are:

- 1. A higher level of management is required to maintain the botanical composition. Fertility and liming practices, seeding rates, frequency and height of defoliation, and choice of species are critical factors in the establishment and maintenance of a desired botanical composition.
- 2. Weed control is more difficult, particularly with annuals.
- 3. Disease problems may be more severe in mixed stands.

# BREEDING FOR COMPETITIVE ABILITY

Breeding programs for species that will be used in mixtures should include mixed stand evaluations during some phase(s) of the programs. However, there are a number of problems regarding mixed stand evaluations which the breeder must consider in selecting genotypes or cultivars for competitive ability.

The question arises as to whether mixed stand evaluations should be included in the selection phase or only in the testing phase of the breeding program. To date, mixed stand evaluations have generally been carried out

only in the later stages of breeding programs to cut down on the number of plants and the amount of time, labor, and land involved. However, to relegate mixed stand evaluation to the testing phase alone, with no prior selection for competitive ability, may result in little or no improvement in competitive ability. Reports vary as to the correlation between performance in pure stand and performance in mixed swards (13,14,16).

Several planting arrangements have been used to evaluate forages in mixed stands. These include broadcast, alternate row, and mixed row plantings. Broadcast and mixed row plantings have generally resulted in higher yields than alternate row plantings (5,9,18,22), although Hanley et al. (11) found no differences between alternate and mixed rows. Lack of uniform competition may pose a problem with broadcast and mixed row plantings. If alternate or mixed row plantings are used attention must be given to the distance between rows, as the effects of competition decrease rapidly with increasing distance between rows or plants within rows (9,22). The use of alternate plants of grasses and legumes or spaced grass plants surrounded by legumes offer alternative planting arranagements, but involve more time and labor.

The performance of forages tested in mixed swards may differ under grazing and clipping. Forages tested under grazing are subject to trampling, soiling, and selective grazing, which are not accounted for under clipping. Bryant and Blaser (4) noted that estimating grazing yields from clipping data may lead to inflation of yields. The bunchy growth habit of annual grasses may cause selective grazing problems in mixed swards (2). To minimize this problem all species included in a mixture should be highly palatable. If plots are to be grazed, frequency and height of defoliation and stocking rates must be closely monitored. As a general rule, frequent low defoliation and high stocking rates leads to clover dominance, while infrequent higher defoliation and lower stocking rates results in grass dominance (2,8,19). However, species vary in their response to grazing (4,15,19).

The nature and level of competition in mixed stands can be manipulated by management practices. The breeder may use optimal management practices where there is only competition for light or he may elect to limit additional factors, such as nutrients and water, in selecting for competitive ability. Legumes have a higher nutrient requirement for potassium, phosphorus, and sulfur than do grasses (2,19). Grasses are highly competitive for these elements, particularly potassium, and deficiencies lead to grass dominance (1,2,8,19). Grasses are generally more tolerant of excesses and deficiencies in soil moisture (2). Rossiter (19) noted that dry years resulted in a higher percentage of grass and wet years resulted in a higher percentage of clover, particularly if rainfall occurred at the time of seeding. High seeding rates of aggressive annual grasses will lead to grass dominance (3,5).

Grass cultivar x legume cultivar (and species) and mixture x environment interactions have been reported for mixed stand evaluations (4,7,9). The grass breeder is faced with question of how many legume varieties and species, years, and locations to use in mixed stand evaluations to provide reliable estimates of competitive ability. This can become very expensive in terms of time, labor, and land, particularly with annuals which must be reseeded each year.

The breeder should consider the specific factors which cause the decline of a species in mixed stands, such as competition for water, light, or nutrients. Many of these problems may be easier solved with pure stands. Several characteristics of annual grasses might be manipulated in pure stands to improve their performance in grass-legume mixtures.

Blaser et al. (1) suggested the use of less vigorous grasses to maintain legumes in mixed swards. Jennings and Aquino (12) stated that characteristics that increase size and vigor early confer competitive ability in rice. Annual ryegrass seedlings are extremely vigorous and aggressive compared to other grasses (1,2,3,6). The rapid germination time and seedling growth rate of annual ryegrass give it an early advantage and may cause it to become the aggressor in mixed swards (2,6). The breeder might consider selecting annual grasses with slower rates of germination and seedling growth, for use in mixtures. Seedling growth characteristics show variation among species and varieties within species (3,6).

A reduction in the capacity of grasses for luxury consumption of potassium would also help to maintain legumes in mixtures (8). Grasses are more competitive for potassium than legumes, although their potassium requirement is lower (1,8,9,19). If grasses germinate faster or initiate growth earlier in the spring, they may cause a potassium deficiency in the legume and thus become the dominant species. Fyfe and Rogers (9) found differences between varieties of tall fescue for their ability to take up potassium.

Alteration of the morphology of the grass plant may offer some possibilities in selection for competitive ability. Jennings and Aquino (12) found that tiller and leaf number, leaf length, spreading growth habit, leaf area index, dry weight, and height were greater in strong competitors. Tall erect bunch grasses tend to be more aggressive than prostrate types under infrequent defoliation (2,8,17). Prostrate types are more competitive under frequent defoliation, probably due to the fact that they are not completely defoliated. Rhodes (17) suggested the development of cultivars with erect tillers and long rigid leaves for systems of optimum defoliation (95-100% light interception). For systems of frequent defoliation he recommended cultivars with prostrate tillers and short leaves. However, Sakai (20) noted that it was difficult to relate competitive ability to any single morphological trait.

Disease resistance is an important trait in breeding annual grasses for use in mixtures. Templeton et al. (21) reported that the environment near the ground in mixed swards was ideal for the development of disease. The resistant species in a mixture will become the aggressor.

Genetic variation for competitive ability exists among species, varieties, and genotypes within varieties (2,3,9,20). Donald (8) noted that competitive ability as an aggregate character is lowly heritable, but that selection for individual factors influencing competitive ability may show much higher heritabilities. Hamblin and Rosielle (10) found that estimates of heritability and genetic variances from mixed stands tended to be unreliable due to competition effects. The breeder who is selecting for annual grass genotypes that will perform well in mixtures is faced with a complex problem. He must decide on the nature and extent of mixed stand evaluations in the selection process, realizing that many factors can influence the performance of mixed stands.

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PANEL DISCUSSION: BREEDING GRASSES AND LEGUMES FOR USE IN MIXTURES

#### PERENNIAL LEGUMES

By W. A. Cope

White clover is an important component of pasture in the USA wherever soil fertility and moisture are adequate. Possibly fifty million acres of pasture have varying amounts of white clover. Red clover and alfalfa are increasingly being used in short term pasture rotations in the upper South. White clover is used almost exclusively in mixture with grasses, while alfalfa and red clover are used predominantly in pure stand for hay.

Basic Requirements of a Competitive Legume. Persistence of the legume in mixture with grass is a major problem. The build-up of disease and insect pests with extensive legume use seems to be the one most significant factor in limiting persistence. However, the basic competitive ability of the legume is important. The giant (ladino) white clover, introduced about three decades ago, is more competitive with the robust pasture grasses than the small white clovers. The term "pasture type" has long been used to distinguish legumes with growth habit conducive to compatability with pasture grasses. Decumbent and small growing types possibly are more tolerant to the selective grazing to which legumes are subjected.

Growth Habit and Pasture Use. Forage legumes differ widely in growth habit. White clover has almost unlimited potential for vegetative reproduction by rooting of stolons. Red clover and alfalfa depend on maintenance of healthy crowns for stand persistence. Birdsfoot trefoil and crownvetch are not extensively grown in the South, but provide examples of the interaction of different growth types when grown with and without grass.

Traits associated with white clover's competitive ability with grasses have been noted by several researchers (1, 5, 11, 12). Gibson (5) summarized such traits with additions from his research:

- 1. Foliage density.
- 2. Amount and time of flowering.
- 3. Number and size of stolons.
- 4. Length of internodes.
- 5. Frequency of stolon branching.

In alfalfa the creeping rooted or broad crowned type has been exploited to develop grazing varieties for semi-arid areas of North America. Hay types of alfalfa may not vary enough in growth habit to differ significantly in grazing performance. In the South alfalfa is used in mixtures with grass only to a limited extent.

Phenotypic variation in red clover has not been extensively used to select for competitive ability with grasses.

Crownvetch is strongly creeping rooted and where adapted could become an important legume. It also varies greatly in stem size and uprightness; these traits could be exploited for grazing.

Birdsfoot trefoil has "pasture types" that have long been recognized. 'Empire' is a naturalized selection from New York State preferred for its survival under grazing. It is smaller and less upright than European varieties.

Interactions: Pure Stand vs Mixed Stand. A number of studies relating phenotype to pasture type have been made with white clover. Knight (10), Dijkstra and de Vos (4), and Gibson et al. (5) noted a strong correlation among genotypes for performance in pure stand and with grass. However, the correlations were not perfect and thus the need for testing genotypes in mixtures. Atwood and Garber (1) noted that the best sods were formed by taller, more spreading, and more densely growing clones, but stated that growth habit of individual spaced plants was not closely correlated with performance in sod. Gibson et al. (5) and Dean (3) each concluded that "non-viney" types are superior in forage production to "viney" types. Gibson (6) found very little difference in varietal ranking of forage production when six white clover varieties were planted with and without grass (Table 1). For individual clones of clover there may be a reversal of performance from pure stand to mixed stand. However, for a variety comprised of many genotypes such a reversal would not be expected.

In its area of primary adaptation alfalfa is often grown in mixture with grass with increased total yield over pure stand. Such plantings may be either mowed or grazed. In the upper South there is increasing interest in sod seeding alfalfa in pasture. Hay types are used. In semi-arid areas broad crowned "creeping" alfalfa is important for grazing. When such types are compared to hay types in mixtures for grazing and in pure stand for hay (9) there may be a sharp reversal in ranking for production and for stand persistence (Table 2). Clearly, the broad crowned types are superior for grazing.

'Penngift' crownvetch yields less (Table 3) under a hay regime than other varieties ( $\underline{2}$ ). However, under simulated pasture conditions ( $\underline{7}$ ,  $\underline{8}$ ) it is more productive and persistent (Table 4). It differs from other varieties in that stems are smaller and less upright.

'Empire' trefoil is more persistent under grazing than the more robust, upright varieties which outyield Empire when managed for hay.

Conclusions. Hay types have been described for various forage legumes, generally in terms of plant morphology. These traits appear to relate primarily to potential for vegetative reproduction--regeneration of independent plants or broadening of crowns. To a lesser extent they relate to tolerance of selective grazing. At present it appears that legume breeders depend to a large extent on selecting in pure stand for traits that contribute to good

Table 1. Yield and rank of white clover varieties grown with and without grass

White clover		nout	Wi	th ass	
variety	Yield	Rank	Yield	Rank	
XPT-1	393	1	646	1	
Ladino	373	2	596	2	
Rega1	368	3	596	3	
Espanso	363	4	546	5	
XPT-2	348	5	548	4	
La. S-1	250	6	512	6	

From Gibson, Crop Sci. 4:344

Table 2. Stand of alfalfa varieties grazed in mixture with bromegrass or cut for hay in pure stand. Final stand in percent of original after five years

Alfalfa variety	Crown type	Final stand in With brome grazed	percent of original Pure stand Hay
Exp. 37	Ъ	105	111
Nomad	b, n	77	106
Rhizoma	ь	77	102
Rambler	b, cr	100	90
Buffalo	n	15	100
Grimm	n	16	100
Ranger	n	21	100
Vernal	Ъ	42	104

From Kehr, Conard, Alexander, and Owen, Neb. Agr. Exp. Sta. Res. Bull. 211, 1963.

Table 3. Forage yield of three crownvetch varieties at Raleigh, North Carolina

	Yield, ton	s per acre
Crownvetch variety	One cut, 1967	Three cuts, 1968
Emerald	0.88	3.99
Chemung	0.87	3.94
Penngift	0.63	2.73

Table 4. Survival of crownvetch varieties grown with fescue and cut at two week intervals for six years

Crownvetch	Stubl	ble ht.
variety	5 cm	10 cm
	crowns	<sub>M</sub> -2
Penngift	78	68
Chemung	51	74
Emerald	10	4

From Hart, Thompson, Hungerford, Agron. J. 69:287.

performance in mixture with grass. Studies on the performance of single genotypes and mixtures of genotypes (e.g., the synthetic variety) when grown both alone and with grass are not very common.

Presently forage legume breeders are faced with the very pressing problem of developing pest resistance. When such problems diminish, greater attention can be given to performance of genotypes in mixtures with grass.

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# PANEL DISCUSSION: BREEDING GRASSES AND LEGUMES FOR USE IN MIXTURES

# BREEDING PERENNIAL GRASSES FOR GRASS-LEGUME MIXTURES

# By R. L. Haaland and C. S. Hoveland

Of the many grass species grown in the United States, three cool-season species (tall fescue, orchardgrass, and Kentucky bluegrass) and three warmseason species (bahiagrass, bermudagrass, and dallisgrass) are major contributors to the forage economy of the Southeast. The cool-season species, especially tall fescue, predominate in the Upper South (KY, TN, VA, and NC) while the warm season species are most important in the coastal plains area. In the Upper South up to one third of the pastures contain adequate legumes but in the Lower South legumes make little or no contribution to pasture production.

There are several reasons why perennial grass-legume mixtures are scarce in the Deep South.

#### **PROBLEMS**

- 1. More compatible grasses such as bluegrass and orchardgrass do not persist in the Coastal Plains.
- 2. Warm-season grass species usually form very dense sods and are extremely competitive.
- 3. The combination of heat stress and multiple pathogen complex severely limit the persistence of cool-season grass and legumes in the Deep South.
- 4. The warm-season grasses are more tolerant than legumes to the pathogen load, heat load, and periodic droughts.
- 5. The positive energetics of  $\mathrm{C}_4$  carbon fixation of warm-season grasses and the negative energetics of  $\mathrm{N}_2$  fixation in legumes gives the warm-season grasses a competitive advantage.
- 6. The grasses can usually withstand overgrazing better than the legumes.
- 7. Grazing animals will often selectively graze legumes leaving the grasses to become more competitive.
- 8. Growing periods of the grasses and legumes often do not nic.
- 9. Grasses may grow up and over clover when forage is allowed to accumulate.
- 10. Grasses will tolerate lower soil pH, P and K thus giving them a competitive advantage over legumes.

Proposed advantages of the grass component of grass legume mixtures have been discussed for many years. They include:

- 1. The grass component, in addition to supplying nutrition, results in a pad for firm footing.
- 2. The fibrous nature of grass roots improves water penetration and percolation in the soil.
- 3. Grasses in a mixed sward will reduce bloat potential.

#### SELECTION CRITERIA AND TESTING

Grass-legume mixtures have been evaluated for years and many management systems have been developed. Little work has been done on developing selection criteria in grasses to make them more compatible with legumes. Grasses and legumes compete in a mixed sward for light, water, minerals, and space. Environmental factors limiting both grass and legumes are pathogen load, heat load and drought. There are many ways breeders might enhance grass compatibility with legumes. For example, more upright leaves would decrease competition for light, pathogen resistance would increase drought tolerance, less luxury consumption of K $^+$  would be beneficial to legumes, less dense sod (less tillering) will decrease space requirements for grass and increase amount of space available to legumes.

For progress to be made in developing grasses that are compatible with legumes the grass breeder must make the following commitments:

- 1. Define objectives associated with compatibility
- 2. Work in close association with legume breeders
- 3. Work in close association with forage managers
- 4. Be innovative

# PANEL DISCUSSION: BREEDING GRASSES AND LEGUMES FOR USE IN MIXTURES

# BREEDING FORAGES FOR USE IN MIXTURES WEST OF THE MISSISSIPPI

# By Ethan C. Holt

While there are hundreds of native and introduced grasses being used for forage in the western portion of the region, breeding programs and/or improved or tame pasture use are limited to relatively few species. Examples of these are: Grasses- Klein, buffel, bermuda, weeping love, old world bluestem, switch, dallis, tall fesue; Legumes- alfalfa, white clover, arrowleaf clover, subterranean clover, sweet clover, crownvetch and vetch.

As the environment (climatic and edaphic factors) becomes less favorable for plant growth and survival, increasing emphasis in plant improvement programs is placed on adaptation, longevity, stand establishment, seasonal growth pattern and forage yield and quality in monoculture. Since essentially no perennial pasture legumes have been developed for the area, no emphasis has been placed on selection criteria for developing forage plants for compatibility in mixtures.

There are opportunities and needs for forage plant mixtures in the area which may consist of perennial grass - annual legumes, perennial grasses and legumes and mixtures of perennial grass species. Problems of such mixtures include:

- 1. Very few adapted, domesticated legumes.
- 2. Alkaline soils leading to minor element deficiencies (esp. iron) in many leguminous species and also in grasses.
- 3. Cotton root rot in many of the central prairie soils, essentially eliminating tap rooted legume species.
- 4. Long periods of drouth, winter or summer, to which warm-season grasses are better adapted than legumes.
- 5. Differences in palatability between species which lead to selective grazing pressure on the more palatable component of the mixture.
- 6. Different components of the mixture may require establishment at different seasons, with the additional moisture, light and nutrient stress as contributed by the competing component.
- 7. Growing periods of components of mixture may not be the same which may be a problem but also may offer advantages.

Advantages of grass-legume mixtures have been discussed by others. There may be advantages to mixtures of grasses, not necessarily the same advantages as for grass-legume mixtures, but with potential needs for screening procedures for compatibility of such mixtures. Among the advantages usually listed are:

- 1. Reduce establishment period prior to utilization.
- 2. Lengthen grazing period and stabilize production.
- 3. Improve forage quality through opportunity for selective grazing.

4. Better animal performance.

The growth and maintenance of species in mixtures may involve several levels and sources of competition and interacting factors, such as:

- 1. Moisture and nutrients, including deficiences of both.
- 2. Physical space, light, temperature, CO2.
- 3. Plant-animal interface including palatability, grazing selectivity.
- 4. Plant response to defoliation.

In the Western portion of the Southern Region, the normal rainfall pattern results in both summer and winter moisture stress periods and the possibility of drouth stress at any time of the year. Summer drouth limits the production of tropical legumes throughout the area and winter temperatures result in winterkill except for a small area in South Texas. Except for alfalfa, perennial temperate legumes are limited by summer drouth. The establishment and winter growth of both perennial and annual temperate species are frequently limited by erratic fall rainfall and winter moisture stress periods. Calcareous soils with limited iron availability frequently result in iron chlorosis in legume species. Thus the first objective in legume breeding programs is adaptation and survival rather than compatibility of legume-grass mixtures.

The environmental limitations described above suggest primarily the use of annual temperate legumes in conjunction with either annual temperate or perennial tropical grasses. The development and use of temperate annual legumegrass mixtures have not encountered any serious compatibility limitations. However, differences in seedling vigor, rate of growth, response to low temperature, length of growing season and response to defoliation are factors which require attention as greater specificity in mixtures and site adaptations develop.

Temperate annual legume-tropical perennial grass associations present numerous compatibility and competition problems requiring solution. These problems are intensified by the extremes in environment encountered in the western area. Fall establishment of the temperate legume is hampered by competition of the perennial grass for space, moisture, nutrients and light. Bunch grasses and open-sod grasses compete less for space and light. Do we breed grasses for these characteristics and for reduced fall growth? Legumes with increased seedling vigor and high temperature tolerance in the seedling stage would seem to be necessary objectives.

Temperate annual legumes are the most competitive for light, moisture and nutrients at the time tropical grasses initiate spring growth. One option would be to develop early maturing legumes or types with open growth to permit initiation of grass growth in the spring. An early maturing legume would make no contribution to the forage quality problem encountered in late spring and summer with tropical grasses. On the other hand, an objective of late legume maturity may not be compatible with maximum summer forage production under conditions of summer drouth. The type of cattle program may influence the type of legume growth pattern required for specific situations.

Grass mixtures are used in the drier areas and these present compatibility problems. Differences in drouth tolerance, earliness, response to defoliation and palatability influence performance and persistence under grazing.

Numerous factors affecting the relationships between or among species

grown in association have been enumerated by individuals on the panel. If breeding programs are to be effective in selecting for mixture compatibility, problems with specific mixtures must be delineated and programs with both or all the species in the mixture coordinated. The question is raised as to whether plant breeding programs in general are sufficiently advanced, refined and coordinated for breeding for specific compatibilities. It would seem necessary to test breeding materials in plant associations if the final product is to be used in mixtures and preferably under grazing.

The development of selection indices for compatibility factors which in turn are influenced by utilization or management factors imposed simultaneously will be difficult. Some of the compatibility problems may be more easily solved by management, especially in situations where intensive management is practical.

### SCLEROTINIA CROWN AND STEM ROT OF ALFALFA IN NORTH CAROLINA

By Ronald E. Welty and Thad H. Busbice

The earliest report of Sclerotinia crown and stem rot as a disease of legumes was in Germany in 1857 (8). The fungus was described, a partial host range, and some control data were published in 1872 (18), but alfalfa was not included as a host until 1915 (10). The disease cycle was described in 1917 (9) and 1919 (23), but it was not until 1965 (22) that the biology of the pathogen was carefully studied and described. The pathogen is widely distributed on forage legumes, but damage to alfalfa is usually less severe than on Trifolium spp., especially crimson and red clovers. Disease losses occur mainly during cool, humid seasons in the southeastern, northeastern, and western United States, and in Britain, Canada, Germany, Norway, and Sweden. The severity of the disease varies from season to season and is often scattered within plantings. Losses may involve entire fields or areas as small as 1-2 cm in diameter. Although plants of all ages are susceptible, the incidence and severity of the disease is greatest in seedlings.

# Causal Organism

The binomial proposed by Eriksson (8), Sclerotinia trifoliorum Eriks., is in wide usage today. According to the Sclerotinia species concept of Purdy (17), the crown and stem rot pathogen is Sclerotinia sclerotiorum (Lib.) d By. (Syn. S. trifoliorum Erik.). Korf and Dumont (14) proposed the new generic name Whetzelinia to replace that portion of the genus which included Sclerotinia sclerotiorum. In this presentation, the synonym S. trifoliorum will be used to designate the causal agent of the disease of alfalfa and other forage legumes.

In North Carolina, apothecia usually develop from sclerotia during cool, wet weather in October and November. Ascospores are carried by wind to leaves and stems and infection occurs when ascospores germinate and penetrate the host directly. Throughout the winter and spring, if high moisture and cool temperatures prevail, secondary infection occurs as mycelium spreads to other leaves and stems. When the food supply is exhausted or environmental conditions are unsuitable for continued growth, the fungus produces hard, black sclerotia on or in stem and crown tissues which remain in soil or on the soil surface. Sclerotia formed in the spring lie dormant during the summer. In the fall sclerotia produce one to several apothecia which contain asci and ascospores. It is generally accepted that mycelium grows only to a limited extent in soil and new plant infections are rarely initiated by mycelium from sclerotia (22).

The effect of temperature on growth and pathogenicity of  $\underline{S}$ .  $\underline{trifoliorum}$  has been well documented (15). The optimum temperature for growth in culture is 15-16 C; the fungus grows between -2 and 27 C and is killed at -24 and

42 C. The effect of relative humidity or free water on infection of alfalfa by S. trifoliorum has not been studied, but Abawi and Grogan (1) concluded for S. sclerotiorum on snap beans that free water for 48-72 hours is required by ascospores for infection and lesion development in beans. Further development of the disease is stopped if the inoculated tissue becomes dry. The same is likely true for alfalfa.

The host range of <u>S</u>. <u>trifoliorum</u> is limited largely to forage legumes and there appears to be little host specificity for isolates. In the greenhouse, isolates of <u>S</u>. <u>trifoliorum</u> from crownvetch and alfalfa are capable of attacking either host (4); in field studies (3), isolates of <u>S</u>. <u>trifoliorum</u> from alfalfa, red clover (<u>Trifolium pratense</u>), and crown vetch (<u>Coronilla varia</u>) are equally pathogenic on these same hosts regardless of isolate source. <u>S</u>. <u>trifoliorum</u> can infect alfalfa, red clover, Ladino clover (<u>T</u>. repens), and crown vetch. Common hosts for <u>S</u>. <u>sclerotiorum</u> do not appear to be natural hosts for <u>S</u>. <u>trifoliorum</u>, however, seedlings of lettuce (<u>Lactuca sativa</u>), tomato (<u>Lycopersicon esculentum</u>), snap beans (<u>Phaseolus vulgaris</u>) and soybeans (<u>Glycine max</u>) can be infected in the greenhouse when favorable conditions are provided for disease development.

The disease can be partly controlled by deep plowing to bury the sclerotia, planting sclerotia-free seed, and maintaining 3-4 year rotations between forage legumes (21, 22). Penta- and tetrachloronitrobenzene (19, 23) and benomyl (13) have been applied to red clover to control crown and stem rot, but the cost of application does not appear to be economical, except perhaps in fields used for seed production. In this study, we applied single and multiple applications of benomyl to alfalfa to evaluate disease control and to better understand the disease cycle. Since it is known that some alfalfa and clover plants or cultivars sustain less <u>Sclerotinia</u> crown and stem rot damage than others (2, 5, 6, 7, 11, 12, 16, 20), but resistance of economic importance is not available (7), we made field evaluations of disease damage in field plants of selected alfalfa cultivars and breeding lines.

Benomyl application. -- 'Team' alfalfa was broadcast seeded on 16 September 1974 on a farm near Raleigh, N. C. After the stand was established, plots  $1.5~\mathrm{m} \times 3~\mathrm{m}$  (5 x 10 ft) were delineated by applying a contact herbicide in 5 cm strips. Six replications of 15 plots were prepared with a 1.5 m border surrounding the experiment. Treatments were assigned to plots in a randomized block design and benomyl at 560 g/935 1/ha active ingredient (0.5 1b/100 gal/A) was applied at 17.2 k Pa (25 psi) once, twice, or monthly. Spray dates were between the 14th and 17th day of each month beginning with October and ending with February. Crown and stem rot damage was determined by counting and measuring or estimating the size of the affected areas as they became visible in each plot. Since 6 plots in each replication were scheduled for spraying during the spring and summer months, but had not yet been sprayed when the disease ratings were made, they were included in the analysis as multiple observations of the nonsprayed controls. Arcsin transformation of the percent damage was used to stabilize the error variance in statistical To determine the influence of monthly sprays on crown and stem rot damage, the means from the nonsprayed controls were compared with the means from plots sprayed once (October, November, December, January, and February), twice (October + December, October + January, and October + February) or monthly (October through February). The F values were used to evaluate the

TABLE 1.--Incidence of Sclerotinia crown and stem rot after applications of benomyl to fall seeded alfalfa

r		Numb	Number of damaged	"	areas <u>1</u> /			1	damaged	area2/	
benomyl applied	1/16	1/21	1/27	m o prors	2/6	2/11	2/12	2/25	or procs. 3/17	3/28	6/4
•											
Monthly	0	0	3	0	0	0	$0.2^{\frac{3}{2}}$	0.2	1.1**	1.7**	**8.0
October	0	T	П	7	3	2	0.0	0.0	15.0	13.8	7.6
November	0	T	0	2	3	3	0.0	0.0	8.7*	11.3	6.7
December	0	0	H	2	2	~	1.1	0.8	8.3*	8.8*	8.0
January	2	П	2	2	2	3	3.6	4.0	27.1	28.1	20.5
February	-	$\vdash$	2	7	9	5	3.1	I.3	26.6	24.1	14.6
Oct. + Dec.	٦	Н	-	7	က	3	0.3	0.0	3.4**	3.0**	2.1**
Oct. + Jan.	0	0	0	3	2	c	1.8	2.2	26.2	21.3	13.7
Oct. + Feb.	2	2	3	2	2	5	5.8*	3.8	21.7	23.9	14.0
Control4/	1.3	1.2	1.3	7	3.5	2	0.7	1.2	25.2	23.6	12.7

1/ Damaged areas were few and scattered when the disease became visible and total occurrence was recorded for each date.

 $\frac{2}{}$  Data transformed to arcsine for analysis following adjustment from cropping history, and then transformed back to percent.

3/ Numbers in same column followed by \* or \*\* are significantly different from the control at P = 0.05 and P = 0.01, respectively.

 $\frac{4}{4}$  Multiple check is the average of six non-sprayed plots per replication.

differences between nonsprayed and sprayed plots at P = 0.05 (\*) and 0.01 (\*\*). The data in Table 1 are the number of areas and the percent crown and stem rot damage in the plots. The percentages were transformed from the arcsin after the analysis.

When symptoms of crown and stem rot were first observed 16 January 1975, damaged areas were 1-2 cm in diameter, and their number and location among plots and replications were highly variable. Subsequent inspections at 3 to 7 day intervals between 16 January and 11 February revealed that the number of circular diseased areas had increased and by mid-February their diameters had increased to 6-8 cm. Subsequently, the size of the diseased area per plot was measured or estimated and converted to a percentage of the plot. The appearance of sclerotia confirmed the damage to have been caused by <u>S. trifoliorum</u>. By the end of March and early April, warm temperatures and long dry periods made conditions more favorable for the host than for the pathogen and regrowth began from nondiseased crown buds.

Significant differences were noted among treatments. The most effective spray schedule was monthly; the most effective single spray was December, followed closely by November; the most effective time for double spray was October + December. Benomyl applied after disease symptoms were observed (Jan. 16) did not reduce further development of damage. In this study, December was apparently a key month to spray for disease control. The relatively high damage on 12 February in plots sprayed in October + February may perhaps be an experimental anomaly because one plot had 60% damage, while the remaining 5 plots averaged 7% damage.

Applications of benomyl before symptoms appeared suppressed the severity of crown and stem rot, but applications after symptoms appeared did little to retard the spread of the disease. Benomyl probably controlled primary (ascospores) but not secondary (mycelium) infection. We did not establish that a single application of benomyl in December can be regularly applied to seedling alfalfa to prevent crown and stem rot, but one or two applications when apothecia are present might give economical control.

Germplasm evaluations.—Alfalfa germplasm developed in several different geographic locations were evaluated by establishing plots in the fall of 1973, 1974, and 1977 in a randomized block design near Raleigh, N. C. The fields were fertilized according to soil test and state recommendations and seeded at 22.4 kg/ha (20 lbs/A). The plots were three drilled rows, 0.76 x 4.57 m, with 22.8 cm between rows and 30.6 cm between plots. The first cultivar test contained five replications of 17 entries; the second cultivar test contained six replications of 23 entries; and the third cultivar test contained six replications of 30 entries. Nine entries were common to the three tests (Table 2). For statistical analysis the square root transformation of the percentage of disease damage was used for test 1 and 2.

After crown and stem rot had been identified, the length of the damaged area was measured in each of the three rows of each cultivar on 8 March 1974 in test one, on 28 March 1975 in test two, and on 15 March 1978 in test three. The amount of damage is expressed as a percentage of the plot. Diseased stems were either cultured or incubated and the development of sclerotia in the stem tissue confirmed the presence of S. trifoliorum. Closely related

TABLE 2.—The amount of Sclerotinia crown and stem rot in several alfalfa cultivars measured in March in three replicated field experiments

Entry	Percentage 1974	of plot damaged b	y Sclerotinia 1978
Liberty (Syn 1)	0.2	8.4	13.4
Liberty (Syn 2) Arc	0.7 2.3	8.5 6.8	5.4 5.7
Team	1.0	7.7	10.2
NCMP 2 (b Syn 1)	0.7	3.3	10.7
NCW 21 (a Syn 1)	0.5	8.9	13.5
Williamsburg	3.2	9.2	12.9
Weevlchek	2.8	13.2	19.8
Kanza Apalachee	5.7 1.1	19.5 3.1	21.6
Victoria	3.9	29.5	-
LSD 0.05 CV (%)	2.87 54	6.51 31	10.85 75

entries are grouped to show similarities in disease incidence. The cultivars tested included the most advanced breeding material in the North Carolina germplasm collection and some were more tolerant to crown and stem rot than others.

To determine whether the apparent tolerance observed in the field could be shown in the greenhouse, 14-day-old seedlings of Apalachee and Victoria, cultivars with the widest range in disease tolerance in 1974 and 1975 (Table 2), were inoculated with oat grains infested with <u>S. trifoliorum</u>. Tolerance was not demonstrated because all plants died from the disease.

The development of cultivars of forage legumes that are tolerant or resistant to  $\underline{S}$ . trifoliorum has been difficult because of the nonspecific character of the pathogen, the lack of a form of resistance easily identified by plant breeders, and an inability to create consistently, in greenhouse and field experiments, epidemics that are similar to those in nature. The nature of the tolerance observed was not determined, but the study does indicate that germplasm adapted to the southeast has morphological or physiological characters that are absent in varieties selected elsewhere. Perhaps tolerance is related to individual plant vigor, as reported for Ladino clover (11).

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# BREEDING FOR PEST RESISTANCE IN RED CLOVER

# By N. L. Taylor and R. R. Smith

Red clover (Trifolium pratense L.) generally has been protected from pests by the use of resistant cultivars. Consequently, much breeding research has been directed by this approach to develop disease resistance and indirectly, to improve yield, quality and longevity. It is the purpose of this paper to examine progress that has been made particularly in the last two decades. Problems and opportunities for further breeding will be elucidated.

#### RESISTANCE TO DISEASES

#### Southern Anthracnose

One of the first diseases of red clover for which resistance was obtained was southern anthracnose caused by *Colletotrichum trifolii* B. & E. This disease occurs in the warmer regions of North America, Kenya and South Africa. Lesions occur on leaves, stems and crowns, causing a typical "shepherd's crook" of the petioles, and eventually resulting in the death of the infected host. Resistance to one race of the fungus is conditioned by one dominant gene according to Athow and Davis (1957). However, genetic studies of resistance are generally lacking. Cultivars which carry resistance to the fungus include 'Kenland' and 'Kenstar'. Perhaps as a result of the use of resistant cultivars, southern anthracnose ephiphytotics have not been observed for several years, at least in Kentucky.

#### Northern Anthracnose

Northern anthracnose caused by Kabatiella caulivora (Kirch.) Karak. has symptoms similar to southern anthracnose except that death of plants usually does not occur directly. The disease is restricted to the cooler sections of North America, Europe and Asia. In recent cool years, the disease has occured in the United States at least as far south as Kentucky. Resistance to the fungus was determined by Smith and Maxwell (1973) to be dominant and controlled by more than three genes. Sakuma et al. (1973) found the resistance was determined by the complementary action of two dominant genes. Resistant cultivars include 'Lakeland' and 'Arlington'. Some of the Northern United States and Canadian cultivars such as 'Altaswede' have a fairly high level of resistance, apparently naturally selected under field conditions over a long period. In Europe, some of the tetraploid cultivars have been reported to possess a higher level of resistance than comparable diploids.

#### Powdery Mildew

Powdery mildew caused by Erysiphe polygoni DC is another disease of red clover which is controlled by using the resistant cultivars 'Arlington', 'Lakeland', 'Orbit' and 'Tensas'. The disease is prevalent wherever red clover is grown. The growth of mycelium and powdery appearance of condia give a conspicuous white or light-gray cast to the leaves which if infection is severe, turn yellow to brown. Quality of foliage is apparently decreased, but no evidence on yield has been obtained. Resistance is dominant in all clones tested, and for five races, resistance was monogenic. For two other races, resistance seemed to be controlled by two genes, and for another race, resistance was inherited in a different manner in different clones. Twelve races of E. polygoni have been identified (Hanson, 1966; Stavely and Hanson, 1967).

#### Rust

Rust, caused by *Uromyces trifolii* var *fallens* produces pustules on leaves, stems and petioles. When infection is severe, pustules are larger and more numerous, causing death and loss of leaves. Rust occurs widely throughout the humid and semi-humid regions of the world primarily in late summer or early autumn. No resistant cultivars are available although breeding for resistance is underway at the Wisconsin station (Engelke, et al. 1975). Sherwood (1957) found 14 plants from 34 cultivars which were resistant to five races of the fungus. Inheritance of resistance was determined by Diachun and Henson (1974a) to be controlled by a single dominant gene. This source of resistance could not be used for cultivar development, however, because it was linked with a seedling lethality factor (Engelke, 1977). Engelke et al. (1975) found that resistance to leaf rust was quantitatively controlled in crosses of some red clover clones.

# Targetspot

Several other leaf diseases occur on red clover, probably the most important of which is Stemphylium leafspot or targetspot caused by Stemphylium sarcinaeforme (Cav.) Wiltshire. It occurs in most humid regions of the world. Lesions on the leaves, stems, and petioles at first are small, irregular dark brown and sunken but later develop into large irregular, dark brown, sunken spots. Several sources of resistance have been isolated (Kilpatrick, 1964; Braverman, 1971) but the inheritance has not been investigated. Methods of screening populations for resistance have been developed at the Wisconsin Station and breeding of a resistant cultivar is underway (Murray et al. 1976).

#### Crown Rot

A very serious disease of red clover which results in death of plants and often complete loss of stands is crown rot caused by *Sclerotina trifoliorum* Erikss. The disease is widespread but apparently is more severe in regions of Europe that have mild winters and heavy snows. In the United States the disease is most prevalent in the southern clover belt including Virginia, Kentucky and Tennessee. Infection first occurs in late autumn when brown spots appear on leaves which drop off and are overrun by white mycelial growth. Infection in the spring results in a soft rot, often under snow cover, resulting in a

dead plant apparent by the time the snow melts or shortly thereafter. Black sclerotia, the resting stage of the fungus, may be found around the base of dead plants. Resistance, but not immunity has been discovered in red clover collected in North Africa (Bond and Toynbee-Clarke, 1967). No reliable seedling selection technique exists, according to Dixon (1975) but Verstad (1960) used a cold frame technique to inoculate seedlings. Inheritance of resistance has apparently not been investigated but is probably quantitative. In field screenings 19 cultivars have been identified as resistant (Dixon and Doodson, 1974). Weibull's 'Britta' is a Swedish crown-rot resistant cultivar (Ludin and Jonnson, 1974). In the United States the cultivar Kenland is reputed to possess a slight degree of field resistance. Autotetraploid cultivars are more resistant to crown rot than comparable diploids according to Verstad (1960). This test was based on chimera plants, i.e. plants with both tetraploid and diploid shoots which were separated clonally and increased to form tetraploid and diploids synthetics. In two cold frame experiments, tetraploids averaged 67 and diploids 54 percent survival 71 to 76 days after inoculation. The effect of induced tetraploidy differed by genotype suggesting that dosage effects of genes for resistance may be important.

## Virus Diseases

Virus diseases are prevalent wherever red clover is grown. The importance of virus in reducing stands and yields is difficult to determine. The most prevalent virus in Kentucky was bean yellow mosaic virus (BYMV), followed by peanut stunt virus (PSV), white clover mosaic virus (WCMV), and tobacco ringspot virus (TRSV) in which were present in 76, 14, 10 and 0.54% of the infected plants examined (Jones and Diachun, 1976). In Wisconsin, BYMV, Wisconsin pea streak virus (WPSV), red clover vein mosaic virus (RCVMV), pea common mosaic virus (PCMV), and alfalfa mosaic virus (AMV) were isolated from 48, 41, 34, 13, and 6% respectively of 187 naturally infected plants. Thirty-nine percent had two viruses, and two percent had three viruses (Stuteville and Hanson 1965). In Sweden, the most prevalent viruses are red clover mosaic virus (RCMV) and red clover necrotic mosaic virus (RCNMV) (Gerhardson and Lindsten, 1937). Another common virus in Sweden is clover mild mosaic virus (CMMV) (Gerharson, 1977). Viruses are transmitted by Acyrthosiphum pisum and Myzus persicae and probably many other species of aphids (Gerharson, 1977). Symptoms of virus vary greatly among clover genotypes within, and among viruses so that cross inoculation and serological tests are necessary for identification. Infected plants may be reduced in vigor to such an extent that death results. Clones are particularly difficult to maintain because of increased opportunity for infection. However, techniques for freeing clones of viruses by meristem tissue culture appear promising (unpublished data, G. Phillips and G. B. Collins, Univ. of Ky). BYMV had no effect on digestibility of clover but increased nitrogen concentration, and decreased chlorophyll concentration and forage yield (Smith and Maxwell, 1971).

Resistance to BYMV, PCMV, and RCVMV was found among breeding lines and cultivars by Stuteville and Hanson (1964). The only reports of inheritance of virus resistance are those of Diachun and Henson (1974b). Clones were selected from the cultivar Kenland which exhibited three types of reaction to BYMV race 204-1: Necrotic local lesion (hypersensitive) reaction inherited as a single dominant gene; resistance to mottling and systemic necrosis inherited as a dominant gene; and a third reaction resistant to mottling again controlled

by a dominant gene which appears to be epistatic to the hypersensitive reaction. No resistant cultivars have been developed although Kenstar and Arlington are reported to have moderate field resistance to BYMV (Taylor and Anderson, 1973; Smith, et al. 1973). At the Kentucky station, research is under way to transfer the hypersensitive reaction to BYMV race 204-1, by backcrossing to the 10 clones of Kenstar.

#### Resistance to Insects

Published reports of resistance to nine insects (Table 1) include leafhoppers, (Empoasca) aphids, weevils (Hupera), the clover root borer (Hulastinus) and Apion spp. As pointed out by Manglitz and Gorz (1972), with the exception of aphids, resistance has occurred largely by chance, probably as a result of natural selection under field conditions. Resistance to the potato leafhopper (Empoasca fabae) is thought to have resulted from natural selection for pubescence (Pieters, 1928). However, it is doubtful that the hairiness of American red clover resulted entirely from selection by the leafhopper in view of the known function of pubescence as a mechanism for high temperature tolerance. Resistance to clover leaf weevil (Hypera punctata) was greater in the Northern United States and Canadian cultivars, Lakeland, Dollard and LaSalle than in the Southern United States cultivars, Kenstar, Chesapeake, Kenland, and Pennscott (Gorz, et al. 1975). Resistance to the alfalfa weevil (H. postica) apparently occurs in most red clover cultivars (Keller, et al. 1970). Bud volatiles of red clover did not attract the clover head weevil (H. meles) as much as those of other clover species (Smith, et al. 1976) although in leaf disc feeding trials, red clover was apparently preferred over four clover species (Smith, et al. 1975). The cultivars 'Manhardy', 'Otten', and 'Altaswede' had moderate levels of resistance to the clover root borer (Hylastinus obscurus) in New York (Gyrisco and Marshall, 1960). Several lines of red clover were found to be slightly resistant to flower weevils (apion spp) (Perju, 1971). 'Dollard' red clover is described as resistant to the pea aphid

Table 1. Published reports of resistance to insects

	Name	
Common	Scientific	Reference
Potato leafhopper Pea aphid	Empoasca fabae (Harris) Acyrthosiphum pisum (Harris)	Pieters, 1928 Markkula, 1970 Wilcoxson, 1960 Gorz et al., 1978
Yellow clover aphid Clover aphid Clover leaf weevil Alfalfa weevil Clover head weevil Clover root borer Clover flower weevils	Therioaphis trifolii (Monell) Nearctaphis bakeri (Cowen) Hypera punctata (Fabricius) Hypera postica (Gyll.) Hypera meles (F.) Hylastinus obscurus (Marsham) Apion spp.	Gorz et al., 1978 El-Kandelgy, 1964 Gorz et al., 1975 Keller et al., 1970 Smith et al., 1975 Gyrisco, 1960 Perju, 1971

whereas 'Wegener' was susceptible (Wilcoxson and Peterson, 1960). The opposite reaction was reported for resistance to the clover aphid (Nearctaphis bakeri) in which Dollard and Lakeland were susceptible and Wegener was resistant (El-Kandelgy and Wilcoxson, 1964). The fecundity of three biotypes of the pea aphid on 10 cultivars of red clover was studied by Markkula and Roukka (1970). All the cultivars were moderately resistant to one biotype, susceptible to the second, and varied from plant to plant within the third biotype. No differences in the resistance of diploid and tetraploid cultivars were found.

The only example of bred insect resistance occurs with the yellow clover aphid (Therioaphis trifolii) and the pea aphid (Acyrthosiphum pisum) (Gorz et al. 1978, by permission). They selected, in five recurrent cycles, for yellow clover aphid resistance and in three cycles for pea aphid resistance under greenhouse conditions. A synthetic, 'N-1' was developed which had resistance to both aphids. In the 5th cycle, 95.6 percent of the plants were resistant to the yellow clover aphid, and in the 3rd cycle, 93.7 percent of the plants were resistant to the pea aphid. Inheritance of resistance was not studied.

# RESISTANCE TO NEMATODES

Other than fragmentary reports of resistance to the root-knot nematode (Ivanoff, 1964; Bain, 1962), most breeding for nematode resistance has been conducted in Northern Europe with the clover stem eelworm (Ditylenchus dipsaci). The nematode causes swelling in the cotyledons, in tissues near the growing point, and in the upper part of the hypocotyl in susceptible plants. Resistant plants are not swollen but exhibit stunted growth. The stands of susceptible cultivars may be eliminated in the seedling year. Personnel of most European countries have tested resistant varieties developed either by natural or artificial selection or a combination of both. In Sweden and Finland, the cultivar 'Merkur' possesses resistance (Bingefors, 1956; Roivainen and Tinnila, 1963). In the Netherlands, only the cultivar 'Flandria' had about the same resistance as Merkur (Dijkstra, 1956) but in Britain several cultivars had about the same or greater resistance than Merkur (Fiddian and Aldrich, 1964). Both tetraploid and diploid progenies were resistant in a test by Toynbee-Clarke and Bond (1970). They also found good correlation between progenies infected as seedlings and progenies infected as one-year old plants. dence of races were found by Fiddian and Aldrich (1964) but Frandsen (1965) interpreted his data to show the existence of races, and suggested that clover breeders should include nematodes from a wide area of clover cultivation. heritance of resistance to stem nematode was studied by Bingefors (1956) by crossing Merkur (resistant) with 'Altuna' (susceptible). Resistance in the  ${
m F}_1$ tended to be intermediate between the parent cultivars and no clear genetic pattern was demonstrated. Nordenskiold (1971) reported that resistance to the nematode was regulated by two dominant genes. One of the two genes was closely linked to the S-locus (self-incompatibility).

# The Root-Rot Complex

Root-rots of red clover are associated with fungi including Fusarium, Trichoderma, Rhizoctonia, Phoma, Gliocladium, Leptodiscus (Elliott, et al. 1969). Non-fungal organisms that attack roots include the clover root borer (Hylostinus obscurus), Sitona sp, and nematodes of various species and genera (Newton and Graham, 1960). The pea aphid (Acyrthosiphum pisum) and the potato leafhopper (Empoasca fabae) feed on above ground parts and with viruses in-

duces stresses which increase root rot. The total group of root-feeding organisms acting together has become known as the root-rot complex, which greatly shortens the life of red clover stands. At any one location, organisms such as the root-borer and Fusarium may be important and lack of resistance is usually considered to be a limiting factor. At other locations where the root borer is not present, Sitona and Leptodiscus spp. are the important agents and lack of resistance to these organisms is considered to be associated with short life of red clover. In addition to stresses imposed by several organisms, flowering and seed production in the seedling year have been shown to be associated with winter injury, and with shortening the life of the stand (Therrien and Smith, 1960; Smith, 1963). On the other hand, Taylor et al. (1962) showed that seed production in the year of establishment of clones was not more detrimental to the stand than forage production provided that plants had the opportunity to develop rosettes before winter. Stand losses and high root-rot incidence in Kentucky occur during the summer months rather than in the winter (Kendall et al. 1962).

It is not surprising that breeding for root-rot resistance has been unsuccessful in view of the complexity of agents involved. In an effort to gain a clear understanding of the situation as it occurs in red clover, Fig. 1 is presented. With a well adapted cultivar (A) and low incidence of physiological hazards i.e. proper management, etc. it is expected that clover, being a perennial will persist for several years as has been shown by Crowder and Echeverri (1961), Crowder and Chaverra (1963) and Gasser and Gagnon (1976). As physiological hazards increase, the persistence decreases. Root-rot organisms will decrease persistence even further. Such a situation occurs where clover is grown in the same fields for many years. With less well adapted cultivar (B), i.e., one that is introduced from another region or country, the decline in persistence is much more drastic particularly so with combination of hazards and root rots. Not illustrated in the figure is the increase in root rots as physiological hazards are increased as shown by Leath and Byers (1973) in which diseased roots were more attractive to root borers than healthy roots.

Breeding for resistance to the root rot complex then may be expected to be effective only by developing broad resistance to wide variety of organisms. Research at the USDA Pasture Laboratory, State College, Penn., on resistance to Fusarium is underway. If this type of resistance conveys resistance to other root rot organisms as well as Fusarium, significant benefit may result. Hybridization of red clover with strongly perennial species which could change the character of the root system thus incorporating general resistance has not been possible to date. The only type of resistance to root rots that exists today is that possessed by a vigorous well-adapted cultivar growing under a minimum of physiological hazards, i.e., ideal management conditions. This is usually termed "field resistance" and is conditioned by a large number of genes low in heritability or by pleiotropic effects.

#### METHODS OF INOCULATION

# Diseases

Details of inoculation methods necessary for development of resistant cultivars are given in Table 2. Satisfactory seedling inoculation methods are available for northern and southern anthracnose, mildew, rust and targetspot. Optimum temperatures range from 20 to 25 C and for all pathogens except that of targetspot, the existence of races has been confirmed indicating that

FIG. I

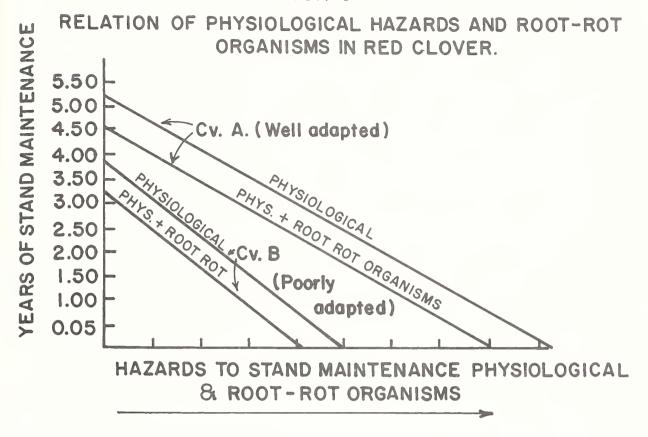


Table 2. Details of inoculation methods for breeding for disease resistance in red clover

	Seedling technique	Optimum temperature	Season	Races identified
		(°C)		
S. anth.	Yes	22-25	Late spring	1
N. anth.	Yes	24	Early spring	18
P. mildew	Yes	24	Fall*	12
Rust	Yes	20	Fall	5
S. leafspot	Yes	20-24	Summer	0
Fusarium	No	28	Summer*	Many species
Crown rot	No		Early spring	0

<sup>\*</sup>Variable.

a wide collection of races from different clover growing areas should be used in inoculation. Unfortunately, the reaction of resistant cultivars in areas other than where they were developed is not well known. For example, mildew resistant Tensas may or may not be resistant in Wisconsin where Arlington is resistant. Because red clover cultivars are heterogenous populations, the reaction of races must be conducted on individual plants as shown by Hanson (1966). The lack of effective seedling techniques for Fusarium and Sclerotinia causes breeding for resistance to these pathogens to be extremely difficult.

Aqueous spore and mycelial suspensions are adequate for the anthracnoses and targetspot but for rust, a 20:1 (w/w) mixture of talc and urediospores is used (Engelke, et al. 1975). The mildew pathogen is also applied dry, usually by placing mildew-infected plants among the population to be selected for resistance. An epiphytolic is developed by rubbing the diseased plants over the test plants several times daily (Hanson, 1966).

For virus epiphytotics, plants may be either exposed to pea aphids taken from infected red clover or peas, or may be inoculated mechanically. In the latter situation, expressed juice from infected plants is merely rubbed on plants to be infected which have been dusted with carborundum. Infection with aphids presents the difficulty of transmitting more than one virus unless extreme care is taken. Also aphid feeding often causes a virus-like symptom when no virus is present complicating the selection procedure (Stuteville and Hanson, 1965). All resistant plants should be examined under field conditions to reestablish the correlation of seedling and mature plant resistance.

## Insects and Nematodes

Inoculation procedures for aphids are greatly simplified as contrasted to other insects because of adequate greenhouse techniques. Entries to be screened for resistance are sown in flats of soil with about 35 to 50 seeds in each of 12 rows per flat. Aphid cultures usually are collected from red clover fields and cultured in growth chambers to eliminate parasites and undesired aphid species. Seedlings at the unifoliolate leaf stage are infested by shaking aphids over the plants in each flat. Aphid infestation may be continued up to two months after which plants are rated on a scale of 1 = highly resistant to 4 = highly susceptible - dead. Only plants in class 1 are retained for propagation. If rescreening is necessary, the surviving plants are cut back, fumigated and reinfested (Gorz et al. 1978, by permission).

In screening for resistance to the stem nematode, infested plants are collected during the summer, cut off ground level and slowly dried at room temperature. Eelworms are extracted by placing infected material in metal sieves which are standing in 8 inch (203 mm) glass funnels lined with milk filters to remove soil particles. A fine mist of water is then provided through four 1-mm aperture nozzles. Water pressure is maintained at 20-25 1b/in (1.4 to 1.8 kg/cm<sup>2</sup>). Eelworms begin to emerge in a few hours and are carried to the beakers below by the flow of water. Eelworm suspension is stored in tapwater at 3 C in a refrigerator before inoculation. are inoculated two to three days after germination. One drop of suspension (approximately 30 eelworms per drop) is placed on each of about 30 seedlings per entry. Inoculated seedlings are then rolled up in chromatography paper (No. 1) and inserted in a bottle without water. High humidity is maintained and after three weeks the seedlings are scored for infection on a scale of 0 = no swelling to 5 = greatly swollen hypocotyl. Seedlings for further breeding are from classes 0 and 1 (Toynbee-Clarke and Bond, 1970).

#### MECHANISMS OF RESISTANCE

Very little published information is available on the mechanisms underlying resistance to red clover pests. Recently, the phytoalexins produced by red clover leaves challenged by various fungi have been cited for a role in inhibiting growth of the fungus. Duczek and Higgins (1976) found that the phytoalexins, medicarpin and maackianin were the only compounds that could account for the inhibition on red clover of Helminthosporium carbonum, a corn pathogen. Biosynthetic pathway studies of these compounds appear to show an isoflavone origin, particularly formononetin which in itself has little inhibitory activity (Debnam and Smith, 1976). The evidence is not clear, however, inasmuch as differences in accumulation, inhibition, and breakdown of the phytoalexins were not enough to explain the difference in pathogenicity of Stemphylium botryosum and S. sarcinaeforme on red clover (Duczek and Higgins, 1976).

Peroxidase activity in hypersensite, BYMV resistant red clover was found to be higher than in susceptible clones indicating a possible mechanism of resistance (Sheen et al., 1975).

No studies were found in the literature concerning the causal mechanisms of resistance to insects or nematodes of red clover.

# Problems in Breeding for Pest Resistance

It is obvious from this survey of research accomplishments that the method used most in breeding for pest resistance is phenotypic recurrent selection. This is not too surprising because the disease resistance obtained is simply inherited, and more importantly, easily recognized, with adequate screening techniques. This is also true in breeding for aphid resistance. Once selections have been made, it remains only to recombine the selected materials by crossing under cages, and one generation is easily cycled per year. Escapes will be eliminated in succeeding generations of selection even though they may be crossed. In most cases, "vertical" resistance appears to be conditioned by dominant genes, and the heterozygote is carried along as resistant. Consequently, the selected strain will never become 100% resistant simply by recurrent selection. This is not of overriding importance, however, and may even be insurance against mutation of new pathogenic races as have occured in self pollinated cultivars. The presence of many cultivars of red clover in different regions of the world also mitigates against this possibility.

While pest resistance selection is simple, it is sometimes more difficult to maintain the desirable characters of a cultivar while increasing pest resistance. If the number of resistant plants in any one cycle is too low, inbreeding depression and loss of vigor may result. The selection of 100 plants results in a theoretical inbreeding coefficient of 0.5% and perhaps represents a practical lower limit of selected plants. Of equal or greater importance is the selection of resistant plants on an annual basis. Some research seems to indicate that such selection may produce more annual genotypes and loss of the persistence characteristic of the modern red clover cultivars. In a backcross program to incorporate mildew resistance at the Kentucky Station, the mildew resistant line was similar to the recurrent parent, 'Kenstar', in all characters except earliness of bloom. Apparently some unintential selection had occurred during the backcross procedure. Earliness in clover has been shown to be associated with lack of persistence (Taylor et al., 1966). More research is needed concerning means of overcoming such selection on an annual basis.

One suggestion is to select only those plants responding to longer photoperiods. These later types should retain persistence.

The problem of races has been referred to earlier, but should be emphaaized again. A broad spectrum of races should be used during the screening process. This may complicate inheritance patterns somewhat but recurrent selection procedures probably will be adequate.

A somewhat more difficult problem is that of non-specific or general resistance which the more adapted cultivars seem to possess. One cannot expect to easily transfer this type of resistance because it is dependent upon many genes, or may be even due to pleiotropic effects. The field resistance to the root-rots, crown rot and perhaps some insects and nematodes may be of this type. If cultivars with this type resistance are transferred to areas where they are unadapted, they may no longer be resistant. Caution should be exercised in attributing specific resistance to a particular disease to a well adapted cultivar. It may be resistant because it is adapted, rather than adapted because it is resistant.

#### SUMMARY

Progress in the development of pest resistance in red clover has been made over the last 20 years particularly with diseases. However, resistances have been combined only in a few cultivars. Much less research has been conducted on insect or nematode resistance. Resistance to specific diseases have been found to be controlled by one or a few dominant genes, but insect resistance inheritance studies are lacking. Most breeding has utilized phenotypic recurrent selection, and in a few cultivars, the backcross method. methods are used because resistance is simply inherited, seedling inoculation techniques are available, and resistance is easily recognized. A more difficult problem is the maintenance of cultivars without change in other desired characters. Little attention has been directed toward understanding mechanisms of resistance to specific red clover pests. Although the root rots have received considerable attention, little progress has been made primarily due to the complex and varied nature of pathogens and parasites on clover roots. Well adapted, vigorous, persistent cultivars possess a broad or field type of resistance which enables them to yield and persist well in spite of root rots and physiological hazards.

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# ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION AND IDENTIFICATION OF FORAGE LEGUME VIRUSES

By M. R. McLaughlin and O. W. Barnett

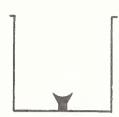
Detection and identification of plant viruses is an essential part of any plant breeding program on virus resistance. This is usually done by combining greenhouse tests involving the inoculation of a series of indicator host plants with serology, electron microscopy, and other laboratory procedures (1). The investment in facilities, equipment, expertise, and time required for successful application of these methods can be a considerable obstacle to plant breeders and plant pathologists working in this important area.

The need for a simple, rapid, sensitive, reliable, and practical means of virus detection and identification prompted us to examine a relatively new serological procedure, the enzyme-linked immunosorbent assay (ELISA) (2). This procedure offers the advantages of specificity, speed, and ease of standardization provided by conventional serological methods, while overcoming problems due to low virus concentrations and particle morphology which often limit the effectiveness of conventional methods (3). The development of ELISA and its application to detection and identification of some forage legume viruses is described in this paper.

## DEVELOPMENT OF ELISA

Labelled antibodies have been used for a number of years to increase the sensitivity of serological procedures in detection of viral antigens. Traditionally, fluorescent dyes and radioisotopes have been used as labels. More recently the use of enzyme-labelled antibodies (4) was shown to be useful in light microscopic detection of viral antigens in tissues. The pioneering work of Engvall and coworkers (5, 6, 7) and Van Weeman and Schuurs (8, 9) demonstrated that enzyme-labelling procedures could also be quantitative and exhibit sensitivities comparable to radioimmunoassay techniques. Engvall and Perlmann first introduced the acronym, ELISA, in 1971 (5). In 1974 Voller et al. (10) adapted ELISA to a microplate method. In 1976, in a review of the theory and practice of enzyme immunoassays in diagnostic medicine, Voller, Bidwell, and Bartlett (11) described the "double antibody sandwich" form of ELISA.

In this method (Fig. 1) specific antibodies are adsorbed to a solid surface in wells of polystyrene microtiter plates (Dynatech Laboratories, Inc.). A test sample suspected to contain the viral antigen is incubated in the wells and any virus recognized by the antibody is bound. Subsequent reaction of the bound virus with enzyme-labelled specific antibody results in formation of the "double antibody sandwich." This complex is then detected by addition of an appropriate substrate with which the enzyme reacts to form a colored product. Qualitative visual ratings or quantitative spectrophotometric measurements can be made of



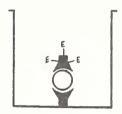
1. Specific antibody adsorbed to polystyrene





2. Addition of test sample Specific antigen (virus) bound by adsorbed antibody.

# RINSE



 Addition of enzyme-labelled specific antibody Formation of "double antibody sandwich"

# RINSE



- 4. Addition of enzyme substrate
  Formation of colored reaction product
- 5. Qualitative visual rating or quantitative spectrophotometric measurement of reaction product

FIGURE 1.--The double antibody sandwich ELISA for plant viruses (11).

the colored product. In the absence of the specific viral antigen in the test sample, the double antibody complex cannot be formed; therefore, no enzyme is present, and no color change occurs upon addition of the substrate.

It was in this form that ELISA was first applied to the detection of plant viruses (2). In 1977 Clark and Adams (3) described the method in detail as it applies to the detection of plant viruses. This form of ELISA has found rapid and widespread acceptance among plant virologists and has been applied to a variety of viruses including: arabis mosaic virus, plum pox virus, strawberry latent ringspot virus, raspberry ringspot virus, hop mosaic virus, prunus necrotic ringspot virus, apple stem grooving virus, and apple chlorotic leafspot virus (2, 3, 12); tomato ringspot virus (13); peach rosette mosaic virus (14); soybean mosaic virus and tobacco ringspot virus (15); apple mosaic virus (16); potato leafroll virus (17); potato virus S and potato virus X (18); prune dwarf virus (19); pea seed-borne mosaic virus (20); cucumber mosaic virus (21); and many others, the reports of which are yet to be published.

## PREPARATION OF ENZYME-LABELLED ANTIBODY

Protein fractions containing specific antibodies were prepared by sodium sulfate precipitation (8). From 1 to 5 ml of antiserum was brought to 5 ml with distilled water, then antibody protein was precipitated by addition of an equal volume of 36% sodium sulfate in aqueous solution. Antibody protein precipitates were collected by centrifugation for 15 min at 6,000 xg, washed once with 18% sodium sulfate in aqueous solution, resuspended in phosphate-buffered saline (PBS - 0.02 M phosphate, 0.15 M NaCl, 0.003 M KCl, pH 7.3) and dialyzed exhaustively against PBS at 4 C. Antibody protein concentrations were estimated spectrophotometrically ( $E_{280nm}^{0.7\%} = 1.5$ ). Volumes of 0.5 to 1.0 ml of antibody protein at 2.0 mg per ml in PBS were reserved for enzyme labelling, while a second portion of the antibody protein solution at 1.0 mg/ml was adjusted to 0.02% NaN3 and stored at 4 C for later use in coating plates. The antibody protein reserved for enzyme labelling was mixed with an equal volume of alkaline phosphatase [EC No. 3.1.3.1, Sigma Type VII, 5 mg/ml in a crystalline suspension of 3.2 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution, pH 7, containing 0.001 M MgCl<sub>2</sub> and 0.0001 M ZnCl2]. The mixture was dialyzed against several changes of PBS at 4 C, then 25% glutaraldehyde in aqueous solution was added to a final concentration of 0.2%, and the mixture was incubated at room temperature 2 hr, then dialyzed exhaustively against PBS at 4 C. The enzyme-antibody conjugate was then dialyzed against 0.05 M Tris-HCI, pH 8 containing 0.15 M NaCl (Tris-buffered saline = TBS). The conjugates were adjusted to 0.5 mg antibody per ml in TBS, made up to final concentrations of 1.0% BSA (bovine serum albumin) and 0.02% NaN3 and stored in the dark at 4 C.

### THE ELISA METHOD

The "double antibody sandwich" form of ELISA (11) was used according to the procedures of Clark and Adams (3) with some modifications. Protein-binding polystyrene microelisa plates (cat. no. 1-223-29, Dynatech Laboratories, Inc., 900 Slaters Lane, Alexandria VA 22314) with flat-bottomed wells were coated with specific antibody by adjusting antibody protein preparations (1.0 mg/ml in PBS) to from 1.25 to 5.0 µg per ml (the optimal concentrations varied between preparations) in carbonate coating buffer (0.05 M sodium carbonate, pH 9.6, containing 0.02% NaN3) and incubating the antibody in the plates (200 µl

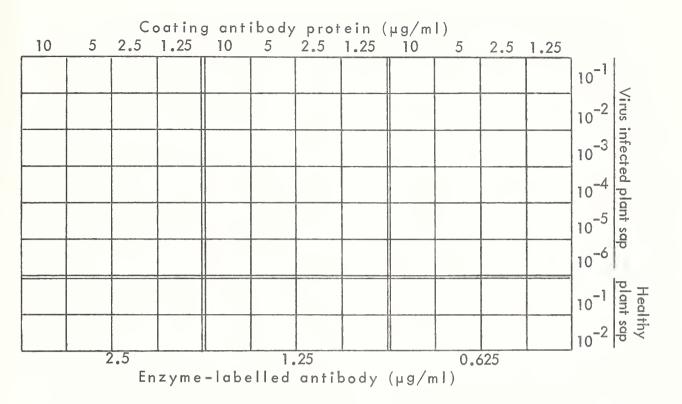


FIGURE 2.—Placement of reactants to determine optimal concentrations for coating and enzyme-labelled antibody.

per well) for 4 hr at 30 C. Nonadsorbed antibody protein was rinsed from the wells by three 3-min washes in PBS containing 0.05% Tween 20 (PBS-Tween). Test samples containing purified virus in PBS-Tween or plant extracts in PBS-Tween containing 2% polyvinyl pyrrolidone (PVP 40,000 MW) were incubated in the plates (200 µl per well) overnight at 4 C. Test samples were rinsed from the wells with distilled water followed by three washes in PBS-Tween. Enzyme-labelled antibody at concentrations of 0.625 to 2.5 µg per ml (the optimal concentrations varied between preparations) was added to the plates (200 µl per well) and incubated 4 hr at 30 C. Unbound enzyme-labelled antibody was rinsed from the wells with PBS-Tween as before and 200 µl of enzyme substrate (p-nitrophenyl phosphate, 5-mg tablets, Sigma Chemical Co.) at 1.0 mg per ml in 10.0% diethanolamine was added to each well. Substrate solutions were incubated in the plates at room temperature for 1 to 3 hr, then 50 µl of 3 M NaOH was added to each well to stop the enzyme-substrate reaction. Dephosphorylation of p-nitrophenyl phosphate yielded a yellow-colored product, pnitrophenol. The presence and intensity of the yellow color was scored visually and/or measured spectrophotometrically. Spectrophotometric measurements were made by diluting the contents of each test well in 1.0 ml distilled water and reading its absorption through a 1-cm light path at 400 nm in a GCA/McPherson Model EU700 spectrophotometer.

Optimum concentrations of coating and enzyme-labelled antibody preparations were determined experimentally according to the design in Fig. 2.

TABLE 1.—Comparison of ELISA detectable dilution end points (DDEP) with infectivity dilution end points (DEP) from alfalfa mosaic, bean yellow mosaic, clover yellow mosaic, clover yellow vein, and white clover mosaic virus—infected plant tissue

Virus (Source plant)	ELISA DDEP	Infectivity DEP (Indicator host)
AMV (white clover)	10 <sup>-5</sup>	10 <sup>-2</sup> (Bountiful bean)
BYMV (white clover)	10 <sup>-3</sup>	$10^{-3}$ (C. amaranticolor)
BYMV (Alaska pea)	10 <sup>-3</sup>	*
CYMV (white clover)	at least $10^{-6}$	10 <sup>-5</sup> (C. quinoa)
CYVV (white clover)	10 <sup>-2</sup>	10 <sup>-2</sup> (C. <u>amaranticolor</u> )
CYVV (Alaska pea)	10 <sup>-3</sup>	*
WCMV (Alaska pea)	10 <sup>-4</sup>	at least 10 <sup>-7</sup> (Bountiful bean)
WCMV (Bountiful bean)	10 <sup>-3</sup>	10 <sup>-5</sup> (Bountiful bean)
WCMV (white clover)	10 <sup>-6</sup>	*

<sup>\*</sup> Not tested.

#### **PROGRESS**

To date we have prepared and tested enzyme-conjugated antibody to alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), clover yellow mosaic virus (CYMV), clover yellow vein virus (CYVV), two isolates of peanut stunt virus (PSV), red clover vein mosaic virus (RCVMV), and white clover mosaic virus (WCMV). With the exceptions of the RCVMV system, which has not been fully examined, and the PSV systems, which showed positive reactions against some healthy plant sap preparations, the ELISA results with these virus systems have been very encouraging.

Comparisons of ELISA detectable dilution end points (DDEP) with infectivity dilution end points (DEP) (Table 1) showed ELISA methods to be at least as sensitive and sometimes more sensitive than conventional infectivity tests, with one exception. In the case of WCMV, a virus which is relatively very stable, reaches relatively high concentrations in infected plants, is very easily mechanically transmitted, and probably carries all of its genetic information in a single particle, infectivity tests proved more sensitive than ELISA. A contrasting situation was observed with AMV, which has a split genome and requires multiple nucleoprotein particles each with a different complement of genetic material to be present at the site of inoculation in order to produce infection (22). All the nucleoprotein components of AMV share common antigenic properties (22), and so it was not surprising that AMV was detected serologically at dilutions where its components were too widely dispersed to cause infection.

In tests using 200-µl samples of purified virus, ELISA detected WCMV in amounts as low as 2 to 20 ng and CYVV as low as 20 ng. Concurrent tests with latex-conjugated antibodies (23) have shown ELISA to be at least as sensitive as latex serology and sometimes 10

to 100 fold more sensitive depending upon which preparations of the respective conjugates were compared.

In tests of field-collected material, 27 clover samples, which had previously been indexed for viruses by inoculation to indicator host plants, were tested with ELISA for BYMV, CYVV, and WCMV. Following host indexing, the samples had been held at 18 C for 4 days and were in generally poor condition when used for ELISA, yet in all but four cases the results of the two indexing methods were in full agreement. In two instances ELISA failed to detect WCMV which the indicator hosts did detect. In both cases the concentration of WCMV in the tissue samples was very low, as indicated by very few chlorotic local lesions on the primary leaves of cowpea test plants. In one case the particular sample had been included in the test because it was almost totally rotted and we wanted to see if it was still usable. In the remaining two cases of nonagreement, ELISA detected BYMV which the indicator hosts did not. In this comparative test, ELISA was often useful in determining whether a particular sample contained BYMV, CYVV, or both. Such distinction could not be made based upon the reaction of the indicator host, Chenopodium amaranticolor Coste and Reyn., because both viruses produced similar necrotic local lesions.

Results of a similar comparative test of 70 field samples representing various clover species showed total agreement between ELISA and greenhouse tests with indicator host plants. Unfortunately for the comparison, both systems indicated no virus infection in any of the samples, making it difficult to evaluate the performance of either method.

## USE OF ELISA IN COOPERATIVE PROJECTS

One of the most encouraging findings concerning ELISA was that microtiter plates could be sensitized with specific antibody and sent via regular mail service to cooperating scientists. The cooperators need only to add the appropriate test and control plant sap samples to the plates, store them overnight in the refrigerator, rinse out the samples with distilled water, and send the plates back by return mail. The ELISA tests may then be completed at the laboratory of their origin. This use of ELISA offers a new dimension in research potential to the plant breeder or plant pathologist who might otherwise not be equipped to do virus detection and identification work, and the opportunity for regional research cooperation is greatly enhanced. A single research center capable of preparing and completing ELISA tests could supply plates to several cooperators, thereby extending virus detection and identification programs throughout the region.

Such cooperative efforts have begun already. Microtiter plates sensitized at Clemson have been sent to cooperators in the S-127 Regional Project on Forage Legume Viruses. The response of cooperators has been positive and early results have been favorable.

## SUMMARY

Enzyme-linked immunosorbent assay (ELISA) is a research tool which offers several advantages.

1. Virus screening tests, which formerly required inoculation of several indicator host plants, took up to three weeks to complete, and required a considerable investment in greenhouse space, may be completed in a matter of hours with ELISA.

2. Comparative tests have shown ELISA to be generally as reliable and often more sensitive than conventional indicator host assays.

3. Sensitized ELISA plates can be mailed to research cooperators who may add test samples and return the plates for completion of testing, thereby opening up new cooperative research potential.

## **ACKNOWLEDGMENTS**

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## COLLECTION OF CLOVER SPECIES IN GREECE, CRETE, AND ITALY

By R. R. Smith, N. L. Taylor, and W. R. Langford

The first phase of a three-phase <code>Trifolium</code> (clover) seed exploration was conducted during June and July, 1977 in Greece and Italy. The initial proposal was drafted by Dr. N. L. Taylor, University of Kentucky on behalf of clover workers in eastern United States. This proposal was submitted to USDA, Science and Education Administration (SEA) Plant Germplasm Coordinating Committee in December of 1975. This proposal was subsequently subdivided into three phases. The first phase was to collect seed and associated <code>Rhizobia</code> biotypes of <code>Trifolium</code> ambiguum M. Bief., <code>T. montanum</code> L., <code>T. noricum</code> Wulf., <code>T. patulum</code> Tausch., <code>T. pignantii</code> Brogn. and Bory., <code>T. pratense</code> L., <code>T. repens</code> L., <code>T. uniflorum</code> L., and <code>T. wettsteinii</code> Dorfl. and Hay. in Greece, Italy and Yugoslavia.

The first phase was approved and funded through the USDA, SEA Plant Germplasm Coordinating Committee. Specific details for conducting this exploration and collection were initiated in December, 1976 through the offices of Mr. Wilfred Phillipsen, Mr. Elmer Hallowell, and Mr. Robert Svec, Agricultural Attaches in Greece, Italy, and Yugoslavia, respectively, and scientists in each country. Contacts and preliminary arrangements were made with the assistance of Dr. George Boudonas, Director, Agricultural Research Service of Greece and Mr. E. Porceddu, National Germplasm Institute of Italy. Attempts were made from the U.S. and through the Agricultural Attache's office to contact appropriate Yugoslavian officials or scientists for assistance. Contact was finally made with the Yugoslavian Ministry of Agriculture through Mr. Svec's office on June 21, 1977 after starting the trip in Athens, Greece. The initial program proposed exploring and collecting in Yugoslavia for seven days, however, it was impossible to firm up details with the Yugoslavian government in time to conduct an exploration there. The seven days programed initially for Yugoslavia were then spent on additional collection sites in Greece.

The exploration trip began on June 20, 1977 in Athens, Greece by the senior author and Dr. W. R. Langford, Director Southern Regional Plant Introduction Station, Experiment, GA. Accessions were collected in Greece from June 21 to July 5, in Crete July 6-8, and in Italy July 12-21. Table 1 has the complete list of accessions of *Trifolium* species collected in Greece, Crete, and Italy during June and July, 1977. All collections were made in native pastures, mountain meadows, natural forests, and undisturbed country roadsides.

## COLLECTION IN GREECE AND CRETE

Detailed arrangements for assistance at each location in Greece were arranged through Mr. Phillipsen and Dr. Boudonas's offices. The route traveled and areas explored in Greece are shown in Figure 1. The central points of operation were Thessaloniki (Macedonia Province), Larisa and Trikkila (Thessali Province), Ioannina (Epirus Province), and Chania, Crete.

TABLE 1. Number of accessions of *Trifolium* species collected in Greece, Crete and Italy during June and July, 1977

	Number <u>a</u> /	of accession	ns from	
Species	Greece	Crete	Italy	Total
alexandrinum	2	_	1	3
alpestre	2	400	- (1)	2 (1)
angustifolium	6	_	1	7
arvense	2	2	1	5
campestre	7	2	4	13
canescens	5	_	15	20
cherleri	2	1	1	4
echinatum	5	_	_	5
fragiferum	2 (3)	2	2 (2)	6 (5)
globosum	1	_	_	1
glomeratum	1	4	_	5
heldreichianum	1	_	- (1)	1 (1)
hirtum	3	_	_	3
hybridum	1	_	4	5
incarnatum	_	_	3	3
ligusticum	_	1	_	1
montanum	_	_	1	1
medium	3	_	7 (1)	10 (1)
nigrescens	4	4	- (1)	8 (1)
pallidum	11	7	_	18
phloeides	2	equin	1	3
physodes	_	1	_	1
pratense	6 (3)	_	33 (1)	39 (4)
repens	28 (4)	4	15 (2)	47 (6)
resupinatum	3	2	_	5
retusum	2	_	_	2
rubens	1	_	_	1
scabrum	4	1	1	6
stellatum	1	2	1	4
spumosum	1	-	_	1
squamosum (maritimum)	4	2	_	6
squarrossum	_	_	1	1
subterraneum	1	2	_	3
suffocatum	_	1	-	1
tomentosum	-	2	1	3
uniflorum	1	_	_	_
unknown 1	4	_	_	4
unknown 2	5	1	_	6
spp.	- (6)	- (2)	- (10)	- (18)
Total no. species	31	18	18	37 <u>b</u> /

 $<sup>\</sup>frac{a}{}$  Value in parenthesis is number of that species with nonviable seed.

 $<sup>\</sup>underline{b}$ / Total number of species. A total of 254 accessions with viable seed and 37 with nonviable seed.

The areas explored in Macedonia Province were around the village of Sere and in the Menikion Mts. (max. 1500-1700 m elevation - area 1), around the village of Beria and in the Vermion Mts. (max. 1700-1800 m elevation - area 2), and central (Polygyras) and western extension of the Khalkidhiki penninsula (max. 400 m elevation - area 3). Species collected in this region are listed in Table 2. Most collections in this region were obtained at elevations greater than 400 m in high mountain meadows being grazed by sheep and goats. The exception to this was along the coast of the central extension of the Khalkidhiki penninsula.

The areas explored in the Thessali Province were the village of Trikkala and Lake Megthobas (max. 1500 m elevation - area 4), the villages of Elasson and Olympia and base of Mt. Olympus (max. 1000 m elevation - area 5), and village of Volos and Pilion Mts. (max. 1000 m elevation - area 6). Species collected in this region are listed in Table 3. This region has a wide range of clover species (19 collected in this area). Generally, the annual species were observed at lower elevations with less rainfall and perennials most frequently observed in the mountains where rainfall was more plentiful. Thirteen collections were made around the village of Trikkila in small native community pastures (elevation less than 100 m). Probably the most productive area of Greece was in the Pilion Mountains near Volos between 650 and 900 m. It was this region where the one collection of *T. uniflorum* was obtained.

The areas explored in the Epirus Province were the route between Larisa and Ioannina to include the village area of Metsovan and the Peristeri Mts. (max. 1800 m elevation - area 7) and the regions around the villages of Ioannina, Delvinaki (max. 1000 m elevation - area 8), and Konista. Species collected in this region are listed in Table 4. All five collection of T. echinatum were obtained in this region at elevations between 500 and 1500 meters. Collections were made in northern Greece within 1 kilometer of the Albanian border.

The western tip of the island of Crete was explored around the villages of Chania, Kastelli, Platanos, and Elos and in the Lefka Ori mountains near the villages of Dmalos (1100 m) and Limni (400 m). Eighteen species of Tri-folium were collected in this region (Table 5). Specimens were very difficult to identify in this area. Most plants were dry with dislodged seed heads. All species collected were annuals except T. fragiferum, physodes, and repens.

## COLLECTION IN ITALY

Detailed arrangements for collection in Italy were arranged in cooperation with National Laboratory of Germplasm in Bari through Mr. Hallowell, Agricultural Attache, office. Dr. Pierluigi Spagnoletti of the Germplasm Laboratory accompanied Smith and Langford on the entire trip providing guidance and translating while collecting specimens for his laboratory. Collections were made throughout the Appennino Mountains through central and northwestern Italy (Figure 2). Collections were also made in the Gargano area (area 1). Eleven species were collected in this region between the 41st and 43rd north latitude region (Table 6). Trifolium species angustifolium, arvense, cherleri, phloeides, scabrum, stellatum, and tomentosum were observed only in this region of Italy. Unlike the pinkish white-flowered collections of T. stellatum obtained in Greece this collection was yellow-flowered. Trifolium species alexandrinum, canescens, fragiferum, hybridum, incarnatum, mantanum, and squarossum were not observed until we were north of the 43° latitude (Table (Continued on page 153.)

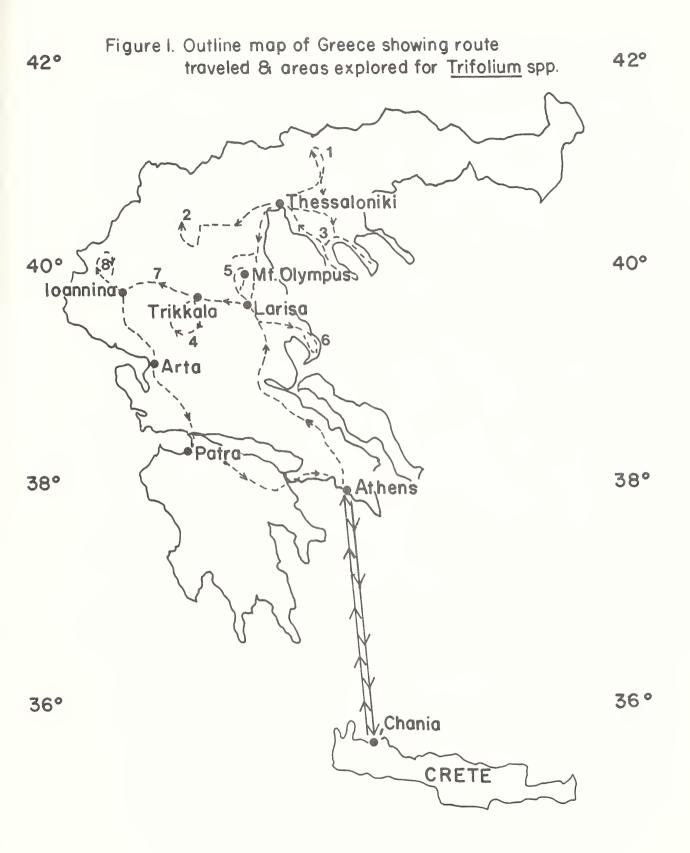


TABLE 2. Trifolium species collected near Thessaloniki in Macedonia Province, Greece

 $<sup>\</sup>frac{a}{}$  Number of accessions in parenthesis.

TABLE 3. Trifolium species collected near Trikkila in Thessoli Province, Greece

 $<sup>\</sup>underline{a}/$  Number of accessions in parenthesis.

TABLE 4. Trifolium species collected near Ioannina in Epirus Province, Greece

angustifolium (1) campestre (3) cherleri (1) echinatum (5) fragiferum (1) hybridum (1)	pallidum (2) pratense (3) repens (6) resupinatum (1) rubens (1) scabrum (1)	stellatum (1) squamosum (1) (maritimum) subterraneum (1) unknown 2 (3)
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 $<sup>\</sup>frac{a}{}$  Number of accessions in parenthesis.

arvense (2) campestre (2) cherleri (1) fragiferum (2) glomeratum (4) ligusticum (1)	nigrescens (4) pallidum (7) physodes (1) repens (4) resupinatum (2) scabrum (1)	stellatum (2) squamosum (2) (maritimum) subterraneum (2) suffocatum (1) tomentosum (2) unknown 1 (1)
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 $<sup>\</sup>frac{a}{}$  Number of accessions in parenthesis.

TABLE 6. Trifolium species collected in central Italy in Appennino Mts. between 43 and 41 N. latitude

angustifolium (1)	pratense (12)
arvense (1)	repens (8)
campestre (2)	scabrum (1)
cherleri (1)	stellatum (1)
medium (1) phloeides (1)	tomentosum (1)

 $<sup>\</sup>frac{a}{}$  Number of accessions in parenthesis.

TABLE 7. Trifolium species  $\frac{a}{}$  collected in central and northeast Italy in Appennino Mts. between 45 and 43 N. latitude

alexandrinum (1)	montanum (1)
campestre (2)	medium (6)
canescens (15)	pratense (21)
fragiferum (2)	repens (7)
hybridum (4)	squarrossum (1)
incarnatum (3)	

 $<sup>\</sup>frac{a}{}$  Number of accessions in parenthesis.

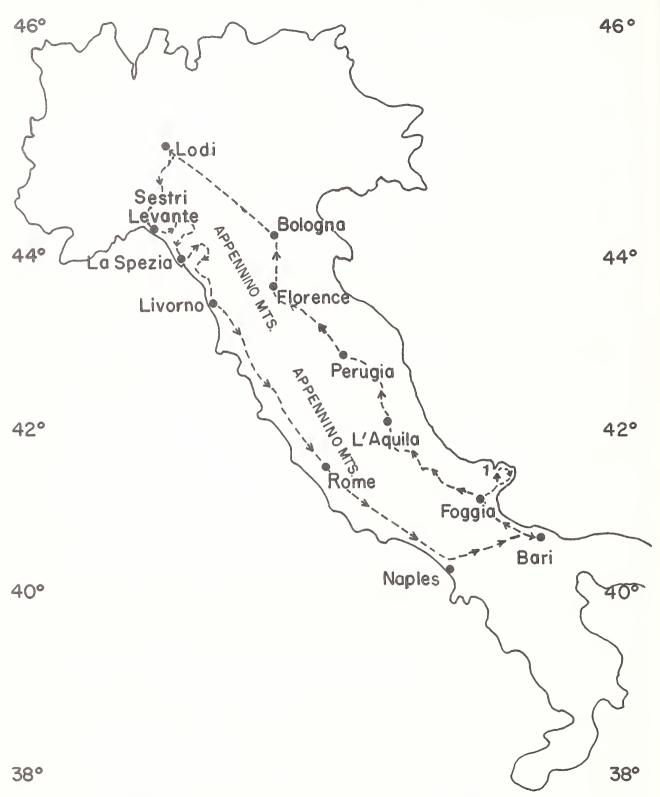


Figure 2. Outline map of Italy showing route traveled & areas explored for <a href="Trifolium">Trifolium</a> spp.

7). Both T. pratense and T. repens were observed throughout the region with T. pratense more frequent at the higher elevations.

#### DISPOSITION OF COLLECTED SAMPLES

Samples were forwarded through the respective agricultural attaches' offices to The Plant Quarantine Station at Beltsville Agricultural Center. After appropriate inspection and clearance the samples were forwarded to the Germplasm Resources Laboratory, SEA, Beltsville, Maryland for assigning plant introduction numbers (P.I.'s) and further documentation. All Trifolium samples were then forwarded to the senior author at the University of Wisconsin, Madison, WI through W. R. Langford. Samples were threshed and cleaned during September, 1977. Approximately eighteen germinated seedlings of each viable species were transplanted to small plastic trays in the greenhouse. Verification of identity of each collection was then made with the assistance of the junior author over the period between December, 1977 to May, 1978. All flowering cross-pollinated species were enclosed in screened cages with honeybees in the greenhouse. Notes such as flowering date, flower color, height, etc. were recorded on each species as they came into flower. Each species that flowered was photographed and a herbarium specimen taken. At the present time seed is being harvested from all species that flowered. Perennials, such as T. repens, pratense, canescens, rubens, alpestre, medium, montanum, heldreichianum, and several unknowns which flowered poorly or not at all were transplanted to the field in May, 1978. Increased seed and remnant original seed will be forwarded to either the Southern Plant Introduction Station (annaul species) or the Northeastern Plant Introduction Station (perennial species).

#### DISCUSSION

Species collected on only the mainland of Greece, the island of Crete, or in Italy are listed in Table 8. T.~pallidum was not observed in Italy while the three collections of T.~incarnatum all came from the central region of this country. The one yellow-flowered T.~stellatun was collected in Italy, but the three pinkish white-flowered accessions were obtained in Greece. Usually the T.~canescens was obtained at high elevations in Italy. T.~cam-pestre and angustifolium could have been collected throughout much of the explored area. T.~campestre was probably the most widely distributed species. Hardly any stop was made without observing a few specimens of this species. T.~repens was probably the second most widely distributed species.

The period between June 20 and July 26, was probably not the most appropriate period for collecting either the annual or perennial species. In general, most of the annual species were very dry and difficult to identify from the plant specimen. Identification in most cases was based on head or seed type. A month earlier (May 15 - June 15) would be the more appropriate period to observe and collect the annuals. On the other hand, many of the perennials were just beginning to flower between June 20 and July 26, making it very difficult to obtain dry seed of these species. Therefore, we would recommend attempting future collection trips to be compatible with either the annuals or perennials. Possibly the perennials might be collected with late annuals.

In addition, sheep and goats had grazed many of the community native pastures and high elevation meadows making it difficult to locate species in

TABLE 8. Trifolium species collected in either Greece, Crete, or Italy

Greece	Crete	Italy
alpestre (2) echinatum (5) globosum (1) heldreichianum (1) hirtum (3) retusum (2) rubens (1) spumosum (1) uniflorum (1) unknown 1 (4)	ligusticum (1) physodes (1) suffocatum (1)	incarnatum (3) montanum (1) squarrossum (1)

 $<sup>\</sup>frac{a}{}$  Number of accessions in parenthesis.

most of these areas. Considerable plant material is available in the areas explored and the grazing problem would not have been as serious if the trip had been earlier.

It was difficult to observe *rhizobium* nodules on most of the dry annuals so no attempt was made to collect them on many accessions. The common species, such as *T. pratense* and *T. repens* were only sampled for *rhizobium* periodically. Collections of bacteria were made on the following species: *T. alexandrinum* (2 samples), *T. campestre*, *T. canescens* (3 samples), *T. fragiferum*, *T. hybridum*, *T. medium*, *T. negriscens*, *T. pratense* (2 samples), *T. repens* (5 samples), *T. squarrosum* (1 sample) and one unknown. Samples were forwarded to Dr. Dean Weber, Cell Culture Laboratory, SEA, Beltsville, Maryland.

Of the initial species outlined in the objectives only *T. pratense* and *T. repens* were observed and collected with any regularity. *T. ambiguum*, patulum, pignantii, and wettsteinii were not collected or even observed in any areas explored. Only one specimen each of *T. montanum* and *T. uniflorum* was observed and collected. *T. montanum* was collected at 800 m elevation along Route 523, five km west of Berceta, north and east of La Spezia, Italy. *T. uniflorum* seeds were collected at approximately 800 m elevation in the Pilion Mountains just southeast of Neohori, Greece. It would appear that we confused the originally identified material as *T. noricum* with *T. canescens*, but an exact identification cannot be made until flowering occurs.

 $T.\ patulum$  and  $T.\ pignantii$  were apparently not observed on this collection trip. Herbarium specimens of these two species would suggest that they may be mistaken for  $T.\ medium$  and  $T.\ rubens$ . Examination of specimens of  $T.\ patulum$  and pignantii collected in the early 1900's located in the National Herbarium, Washington, D.C. provide evidence to support this similarity between these species.

While we attempted to maintain a rigid watch for the species listed in the objective and even with the excellent guidance and assistance provided we were disappointed in the actual number of collections made of the desired species. Contact is being maintained with personnel in both Greece and Italy who will continue the search. In addition, future trips should include Yugo-slavia, if at all possible. For the best cooperation and assistance it is recommended that such a trip be of mutual benefit to both countries. The past trip was mutually beneficial in that many ideas, and in a few cases, germplasm was shared by members of both countries.

#### SUMMARY

During the period of June 20 to July 26, 1977 thirty-seven species of the genus <code>Trifolium</code> represented by 291 accessions were collected throughout <code>Greece</code>, <code>Crete</code>, and <code>Italy</code>. In <code>Greece</code> and <code>Crete</code> collections were made from sea level to elevations of 1800 meters. Samples were collected in the Menikion and Vermion Mountains and the <code>Khalkidhiki</code> region in the Macedonia Province of <code>Greece</code>. In Thessali Province samples were collected in the vicinity of <code>Trikkila</code>, <code>Lake</code> Megthobas, the base of <code>Mt</code>. Olympus, and Pilion Mountains. Collections were made in the <code>Epirus</code> Province near the border of Albania and in the vicinities of <code>Ioannina</code> and <code>Metsovan</code>. Fourty-one samples were collected in the western one-fourth of <code>Crete</code>. One hundred-one samples were collected along the eastern slopes of the Appennino Mountains from near <code>Bari</code> to the Po Valley. One day was spent exploring the <code>Gargano</code> area east of <code>Foggia</code>. The last three days of travel were spent in the mountains northeast of <code>Genoa</code> traveling in a southeasterly direction toward <code>Rome</code>. This latter region had the greatest diversity of perennial <code>Trifolium</code> species of any area explored.

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# RECENT DEVELOPMENTS IN BREEDING AND SELECTION OF TROPICAL LEGUMES (STYLOSANTHES) FOR THE DEEP SOUTH

## by J. B. Brolmann

The first tropical legumes for use in Florida pastures were introduced during the 1950's. Extensive testing of many introductions, in pure stands and in combination with grass, followed. Encouraging results have been obtained with the genera Stylosanthes, Desmodium and Siratro (5).

Selection and breeding of warm-season legumes have been conducted in Australia. Important selections from plants introduced from other areas of the world were made in the 1950's and 1960's. Most of the introductions were from South America where over 4,000 species of Leguminosae are found. The most useful genera were Centrosema, Desmodium, Stylosanthes and Macroptilium. The large diversity within Stylosanthes has made it possible for Australian workers to make rapid progress (4). Most commercial tropical forage legume varieties in Australia have been a result of their very elaborate plantintroduction program. A few varieties, such as Siratro (Macroptilium atropurpureum), however, were the result of their breeding program.

Growth of most tropical legume species suitable for forage is reduced by cold weather. Frosts damages or kills vegetation. Frosts do not normally occur until December in South Florida. Re-growth occurs with warm weather in the spring. To bridge this gap in forage production, more cool-temperature-tolerant warm-season legumes need to be developed. Obtaining plants with cold or frost-tolerance (from higher altitudes or greater latitudes in South America) is of particular importance.

In south Florida, as in most sub-tropics, pasture growth is limited by drought or flood at some time during the year. Development of varieties which persist under these conditions is an important goal.

In Florida emphasis has been placed on selecting within the genus Stylosanthes. This genus is found in both the wet and dry tropics. It grows under various soil conditions and has large morphologic and genetic differences. The variability within Stylosanthes species is very desirable for selection or breeding purposes. The group contains cross-pollinated as well as self-pollinated species. Open-pollinated clones of S. guianensis produced progeny yielding 3 to 4 times as much dry matter as progeny from self pollinated clones (1). Selfing resulted in inbreeding depression in this case. Some species like S. hamata are predominantly self-pollinating. Interspecific crosses could serve as means of variety improvement. Natural hybrids occurring in older field plots, the result of interspecific crossing, have been found and are being evaluated.

Native Stylosanthes hamata occur on the east coast of Florida from Jupiter south, on calcareous soils. There are a variety of ecotypes, including diploids (2N=20) and tetraploids. Recent investigations indicate that tetraploid  $\underline{S}$ . hamata are more vigorous than diploids (2). Tetraploids grow at lower pH than the diploids. When tetraploids are open pollinated, natural crosses with other species may occur in the field, sometimes producing vigorous interspecific hybrids. The F-2 consists of a great variety of types which can be selected for desirable agronomic qualities. Breeding lines are

screened by various selection pressures for persistence under sub-optimal conditions such as flooding and freezing. Flood tolerance of several Stylosanthes sp. has been tested and results indicate that only a few accessions are tolerant to flooding. S. erecta and some Stylosanthes hybrids will tolerate flooding for several months under controlled conditions, but growth is usually reduced (3). Frost will kill top vegetation of all Stylosanthes. Most accessions however, will regenerate from the crown. Some species like S. erecta and S. macrocarpa will regenerate from roots after frost. One accession of S. macrocarpa and two accessions of S. montevidensis survived the severe 1976-77 winter in central Florida (26 nights with frost).

In field tests early flowering, low seed producing accessions of  $\underline{S}$ . guianensis when grown in Bahia were far more persistent than the late flowering, high seed yielding ones.

The great diversity of types in the genus <u>Stylosanthes</u> offers a good possibility of developing varieties for almost any tropical or subtropical environment. Further testing of advanced breeding lines should be encouraged in other areas of the south. The use of tropical legumes is still very limited in South Florida. There is a growing interest, however, to extend their use and to find varieties suitable for Florida conditions.

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